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OIL FROM SEED KERNEL OF PRUNUS PERSICA

K. L. Dhar and R. N S. Chauhan
Chemistry Department, Th. D S B Govt College Naini Tal

Oil from the seed kernel of *Prunus persica* consists of 5 8% solid fatty acids and 94.2% of liquid fatty acids out of a total fatty acid content of 90 5% of the oil. The unsaponifiable matter from the oil (1 11%) contains sitosterol. Further work of the component acids of the oil is in hand

Prunus persica [N O Rosaceae Aru (Hindi, Urdu Kumaoni) peaches (English)] is a tree of variable size. Leaves appear after or with the flowers and are variable in shape and size in different varieties but generally they are oblong, laneaceolate, serrate petiole glandular Flowers are pink which turn white at the time of drooping Fruits are variable in size and shape. Flesh soft, white or yellowish green and sometimes covered with fine paste. The oil has got some medicinal value and is used along with hydrocotyl plant for a type of fever

Analysis of Peaches Oil

The aim of this work was to analyse the oil from peaches of Kumaon region for which no analytical data is available. The oil has got some medicinal value.

In the present work the seeds obtained locally have been investigated. The kernel, amounting to about 8.9% of the seed, when extracted with petroleum ether gave 43.6% (of the weight of kernel) of a light pale yellow colour oil without any odour The physical and chemical characteristics of the oil are shown as under —

Specific Gravity	0.9275
Refractive index	1 4705
Viscosity	43.8
Acid value	9.5
Saponification value	190 4
Iodine value	
(Rosenmund Kuhnhenn s method)	85.38
Richert Miesel Value	0 056
Unsaponifiable Value	1 11 %

Component Fatty Acids The mixed acids obtained from the oil by saponification with alcoholic potash was subjected to Twitchall's separation and resolved into 5.8% solid and 94.2% liquid acids.

Unsaponifiable matter The unsaponifiable matter (1 11 %) obtained from the oil was tested for the presence of sterol by Liebermann Burchard reaction when it gave violet colouration. Identification of the sterol was effected by first getting an addition product with digitonin and subsequently treating it with acetic anhydride when the addition product broke down into sterol acetate, and digitonin (Jamieson, *loc cit* p 417) The acetate after crystallisation from absolute alcohol melted at 125° C indicating it as sitosterol acetate

Experimental

Extraction of oil The extraction of the oil was done by the use of organic solvents. Petroleum ether (B P 60°-80° C) gave maximum yield. Powdered seeds were extracted in portions of 100 g with petroleum ether in a glass Soxhlet for 12 hours. The extract was dried over anhydrous magnesium sulphate and filtered. On removal of the solvent completely under reduced pressure 43.6 gms. of oil was obtained. The oil was then treated with animal charcoal and fuller's earth to give a clear light pale yellow oil This was used as such for other experiments.

Saponification of the oil 108.42 g of oil was taken in 1 litre flask with 28 parts by weight of KOH in about 500 parts of alcohol (98%) The contents were boiled under reflux condenser for 3 hours. After refluxing water was added to keep KOH concentration moderate. About half of the alcohol was distilled off Excess of water was added and then cooled and transferred to a separating funnel and extracted with ether to remove unsaponified water The aqueous solution on acidification with dil H_2SO_4 (40%) was steam distilled (for 3 hours) to get volatile fatty acids. The oily layer in flask was extracted thrice with ether and the ether was distilled off to give 98.194 g of fatty acids.

Separation of Fatty Acids

The above mixed fatty acids (98.194 g) was subjected to Twitchell's lead salt separation using 70 g of neutral lead acetate and 500 c.c., of ethyl alcohol (95%) and separated into 5.694 g of solid (saturated) and 92.50 g., liquid (unsaturated) acids.

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A NOTE ON TWO SPECIMENS OF *HEMIDACTYLUS FLAVIVIRIDIS* RÜPPEL WITH BIFID TAILS

B. P. MEHRA

Division of Entomology, Indian Lac Research Institute, Namkum.

On November 5 1959 the author collected a bifid tailed house-gecko from his residence at the Indian Lac Research Institute. On 10th April 1961 he collected another specimen of a bifid tailed house-gecko from the laboratory of the Institute.

Lizards with a bifid or a trifid tail have previously been recorded by Brindlay (1894) Brindlay (1898) Stuart (1908) Loveridge (1920) Loveridge (1923) Hora (1926) Das (1932) and Mahendra (1936)

In both the specimens the bifid portion commences a short distance before the free end of the tail and in both the specimens the forking is in the horizontal plane i. e., the two branches of the bifid growth are right and left For brevity the two specimens of Gecko will be referred to as A and B

SPECIMEN A (FIG. 1)

(a) *Measurements*

Length from snout to cloaca	—6·43 cm.
„ „ cloaca to fork of bifurcation	—5·00 cm.
„ „ fork to tip of long branch	—1·65 cm.
„ „ „ short branch	—1·40 cm.

The portion of the tail between the cloaca and the commencement of the fork of bifurcation is uniformly divided into seventeen segments, the first one being 0 5 cm. in width.

The long branch narrows suddenly first at 0 6 cm. from the fork and then at 0 85 cm. from this point. That is, it can be divided into three segments. The short branch narrows suddenly at 1 0 cm. from the fork.

(b) *Scaling*

The first segment next to cloaca is covered with small scales while in the rest of the sixteen segments the mid ventrals are laterally elongated flanked by smaller scales which gradually merge into lateral scales.

The ventral scales in the first two segments of the long branch are elongated laterally and arranged in a single row touching the lateral scales. The ventrals in the first segment are uniformly broad while in the second segment they are narrower towards the proximal end and broader towards the distal end. In the third segment the ventrals are small and haphazardly arra

The ventral scales in the short branch are arranged in a row touching the laterals in both the segments except that those in the second segment are narrower than in the first.

Dorsally both the branches are uniformly covered with the usual small scales

(c) *Colouration*

Dorsally the two distal segments of the long branch and the whole of the short branch is of a lighter colour than the rest of the tail which is of grey colour Ventrally the whole of the tail including the two branches, is of uniform cream colour

SPECIMEN 'B (FIG 2)

(a) *Measurements*

Length from snout to cloaca		—7 3 cm.
"	cloaca to fork of bifurcation	—5 3 cm.
	fork to tip of long branch	—2·15 cm.
	short branch	—1 15 cm.

There is an injury in the long branch at 0·55 cm. from the fork. The portion of the tail between the cloaca and the fork can be divided into three distinct regions

Cloaca to commencement of segmented region	—0·8 cm.
Segmented region	—1 4 cm
Unsegmented region	—3 1 cm

The segmented region consists of four segments.

(b) *Scaling*

The first region is ventrally covered with small scales.

In the segmented region the mid-ventrals are prominently big, broader than long and distinctly divided into two halves by a median longitudinal depression. The mid-ventrals are flanked by smaller scales which merge into the laterals

The mid ventrals in the third region are longer than broad, flanked by smaller scales which merge into the laterals.

The mid-ventrals of the last region continue in the longer branch without any demarcation The small scales flanking the mid ventrals gradually disappear towards the free end

Dorsally the long branch is uniformly covered with the usual small scales

The short branch is uniformly covered with small scales alround, which are smaller than those on the dorsal side of the long branch

(c) *Coloration*

Dorsally both the branches are of the same grey colour as the rest of the tail. Ventrally also they are of uniform grey colour as dorsally except the portion of the long branch between the fork and the injury which is lighter in shade and a continuation of the rest of the ventral tail.

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The ventral scales in the short branch are arranged in a row touching the laterals in both the segments except that those in the second segment are narrower than in the first.

Dorsally both the branches are uniformly covered with the usual small scales.

(c) *Colouration*

Dorsally the two distal segments of the long branch and the whole of the short branch is of a lighter colour than the rest of the tail, which is of grey colour Ventrally the whole of the tail, including the two branches, is of uniform cream colour

SPECIMEN 'B (FIG. 2)

(a) *Measurements*

Length from snout to cloaca ..	.. —7 3 cm.
„ „ cloaca to fork of bifurcation	—5 3 cm.
„ „ *fork to tip of long branch*	.. —2·15 cm.
„ „ short branch	... —1 15 cm.

There is an injury in the long branch at 0·55 cm. from the fork. The portion of the tail between the cloaca and the fork can be divided into three distinct regions

Cloaca to commencement of segmented region ...	... —0·8 cm.
Segmented region	—1 4 cm.
Unsegmented region ..	—3 1 cm.

The segmented region consists of four segments.

(b) *Scaling*

The first region is ventrally covered with small scales.

In the segmented region the mid-ventrals are prominently big, broader than long and distinctly divided into two halves by a median longitudinal depression. The mid ventrals are flanked by smaller scales which merge into the laterals

The mid-ventrals in the third region are longer than broad, flanked by smaller scales which merge into the laterals.

The mid ventrals of the last region continue in the longer branch without any demarcation. The small scales flanking the mid ventrals gradually disappear towards the free end.

Dorsally the long branch is uniformly covered with the usual small scales.

The short branch is uniformly covered with small scales alround, which are smaller than those on the dorsal side of the long branch.

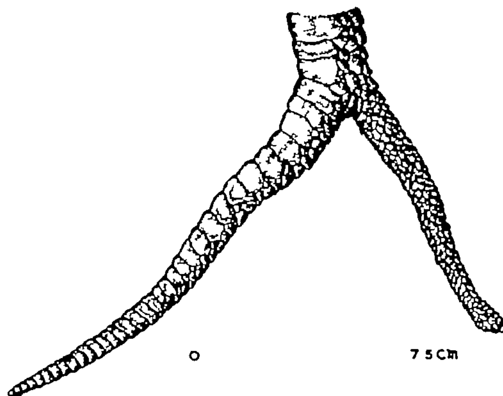


Fig. 1 Ventral aspect of the bifid portion f the tail of *Hemidactylus flaviviridis* Rüppel (Specimen B)

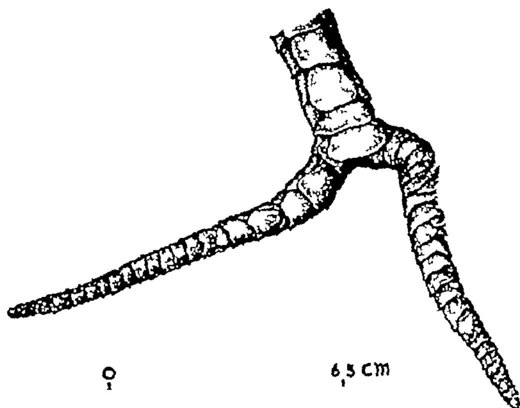


Fig. 1 Ventral aspect of the bifid portion of the tail of *Hemidactylus flaviviridis* Rüppel (Specimen A)

OIL FROM SEED KERNEL OF PRUNUS ARMENIACA

K. L. DHAR AND R N S CHAUHAN
Chemistry Department, Th. D S B Govt College, Naini Tal

Oil from the seed kernel of *Prunus armeniaca* consists of 4 99% of solid fatty acids and 95 01 % of liquid fatty acids out of a total fatty acid content of 90.2% of the oil. The unsaponifiable matter from the oil (1.09%) contains sitosterol. Further work on the component acids of the oil is in hand

Prunus armeniaca [N O Rosaceae. Khubani (Hindi, Urdu Kumaoni) Apricot (English)] is a tree of variable size. The leaves are broadly ovate, petiole, glandular Flowers are pinkish or nearly white. The seed kernel is of two kinds in one case it is bitter and is inedible, while in the other it is edible resembling almonds in taste. The oil has got medicinal value and is generally used in earache and massage. The oil is being exported to U.S A. in large quantities but specific uses to which it is put there are not known.

Analysis of apricot oil has been reported by Hilditch from other parts of the world. The aim of this work was to analyse the oil from apricot of Kumaon region for which no analytical data is available. As expected the composition of the oil investigated by us differs appreciably from those that have been reported

In the present work the seeds obtained locally have been investigated. The kernel, amounting to about 38 / of the seed when extracted with petroleum ether gave 33.3% (of the weight of the kernels) of a pale yellow oil with a characteristic agreeable odour It could not be decolourised by ordinary chemicals. The physical and chemical characteristics of the oil are shown as following :—

Specific Gravity	0 9281
Refractive index	1 4701
Viscosity	36 80
Acid Value	1 07
Saponification value	189 20
Iodine Value (a) Wijs	78.1
(b) Wijs (Modified)	85 0
(c) Hanus	87 3
(d) Rosenmund-Kuhnhenn	87 2
Richert-Miesel Value	0 13
Unsaponifiable Value	1 09

Compound Fatty Acids The mixed acids obtained from the oil by saponification with alcoholic potash was subjected to Twitchell's lead salt separation and resolved into 4 99% solid and 95 01% of liquid acids.

EFFECT OF METALLIC CONTAMINATION AND STORAGE TEMPERATURES ON THE KEEPING QUALITY OF KHOA

A. JALIL, N N PANDIT AND S N SINGH
Department of Animal Husbandry & Dairying B R. College, Agra

Milk—a perishable article of diet is converted into various milk products, viz., *ghee dahi* butter *channa*, *khoa* and other condensed and powder forms In Indian villages most of the surplus milk is converted into *ghee* and *khoa* The estimated output of *khoa* in Indian Union is about 4·9 million maunds annually which accounts for about 4 3 per cent of the total milk production (Report on Marketing of Milk 1956) It is an unfermented milk product concentrated to 70-75 per cent total solids by evaporation of water Its nutritional significance is due to its high level or proteins,—rich in tryptophane and lysine—the two very essential amino acids, to its high calorific value ranging from 1800-2337 calories/lb (De and Ray 1952 and Warner 1951) and to its richness in minerals and fat soluble vitamins.

The quality of such an important Indian dairy product varies considerably from place to place depending upon a number of factors, such as quality of milk used, metals coming in contact during preparation adulterants, duration and condition of storage—to mention only a few The most serious limitation in the marketable and keeping quality of *khoa* develops from dirty and rusty *karahis* and the practice of wrapping the product in green tree leaves or dirty cloth accompanied by different malpractices followed to adulterate the product at the cost of consumers It is not uncommon to find variety of samples of *khoa* in the same market at the same time with widely differing chemical, physical and microbiological characteristics. Though known for a long period not much work has yet been done on the effect of various factors influencing the quality of this product. Dave (1940) has thrown some light on the method of *khoa* preparation in Gujerat and his views, in conformity with those of Davies (1938) suggest some improvements in the art of *khoa* manufacture. Bhatt, Khorshed, Sethna and Fernandies (1949) have carried out researches on the chemical and microbiological studies of *khoa* and are of the opinion that it can last for 48 hours under cold storage conditions Irradiation by different rays, they say is a modern method for preserving its keeping quality for longer durations of time. Iyer Kannan and Basu (1948) while studying the composition of this product have unearthed some relationships between the original composition of milk and the final product prepared from it. De and Ray (1952) have worked out the ratio of S N F/fat which influences both its yield and moisture content. Further these workers can be credited to have

conducted some investigations on the shelf life of this product and the changes it undergoes during storage.

Contemplating the meagre information available on the subject an investigation was planned to study the effect of metallic contamination, the storage temperature and the wrappers used on the keeping quality of *khoa*.

Materials and Methods

Fresh, mixed buffalo milk samples standardised to 6·0 per cent fat were converted to *khoa* using different metal pans,—stainless steel brass, aluminium tinned brass and iron. Every possible technological precaution was taken during its manufacture and a uniform time/temp. relationship ensured. The individual samples were halved and each half triplicated to be wrapped in ordinary butter paper sodium propionate deterged butter paper and sterilized paper wrappers. Half of the samples were stored at room temperature and the other counter part put in a refrigerator at $5^{\circ} \pm 1^{\circ}C$. The samples were tested both while fresh and during storage for different organoleptic, chemical and physical constants. The methods of analyses were adapted from "manual of butter industry for organoleptic constants and Association of official Agricultural chemists (1949) for different chemical and physical constants.

Results and Discussion

A study on the keeping quality of *khoa* manufactured under standard conditions with a few variables like metals, wrappers and storage temperatures was envisaged The results obtained are discussed in the following pages. While fresh, almost all the samples with light greenish white appearance, soft loose body and smooth texture showed an insignificant variation in different physical and chemical constants. However acidity percentage (Table 1 and Fig 1) and the peroxide values (Table 2 and Fig 2) were comparatively lower in samples prepared in stainless steel than iron *karahis*

TABLE 1

Showing the percentage acidity at different storage periods in khoa prepared in different metal pans

Metals	Storage Period at Room Temperature in Days (A)				
	1	3	5	7	10
Stainless Steel	0 090	0 100	0 133	0 630	0 833
Aluminium	0 100	0 110	0 150	0 676	0 926
Tinned Brass	0 100	0 130	0 396	0 736	0 9 6
Brass	0 100	0 120	0 386	0 770	1 000
Iron	0 110	0 130	0 476	0 860	1 300

(Continued on page 15)

TABLE 1—(*Contd.*)

Showing the percentage acidity at different storage periods in khoa prepared in different metal pans

Metals	At Refrigeration Temperature in Days (B)				
	1	7	15	20	25
Stainless Steel	0 090	0 115	0 203	0 495	0 706
Aluminium	0 100	0 125	0 233	0 636	0 770
Tinned Brass	0 100	0 125	0 223	0 606	0 796
Brass	0 100	0 120	0 225	0 595	0 830
Iron	0 110	0 150	0 256	0 630	1 030

The peroxide values ranged from 1 4 (stainless steel and tinned brass) to 1.5 (remaining metals) while acidity was found to range from 0.090 to 0 110 per cent respectively

TABLE 2

Showing the peroxide value at different storage periods in khoa prepared in different metal pans

Metals	Storage Period at Room Temperature in Days (A)				
	1	3	5	7	10
Stainless Steel	1 40	4 40	5 70	11 50	14 60
Aluminium	1 50	4 95	8 45	13 50	17 30
Tinned Brass	1 40	4 90	6 86	14 20	17 82
Brass	1 50	5 03	7 50	12 80	15 73
Iron	1 50	6 90	9 53	12 80	18 28

Metals	At Refrigeration Temperature in Days (B)				
	1	7	15	20	25
Stainless Steel	1 40	2 76	4 76	6 33	8 73
Aluminium	1 50	2 16	4 66	7 93	10 10
Tinned Brass	1 40	2 56	3 46	8 23	9 76
Brass	1 50	2 86	5 00	7 73	10 28
Iron	1.50	3.36	5 56	8 63	12 53

During storage, an appreciable difference in the keeping quality of *khoa* prepared in different metals was noticed This difference widened in the samples stored in unsterilised wrappers (Table 3 and Fig 3) The characteristic physical symptoms of spoilage were profuse mould growth bleaching appearance and white spots. The resistance to spoilage was highest in samples prepared in stainless-steel pans followed by aluminium, brass, tinned brass and iron. At room temperature samples from stainless-steel were found to be edible upto 6 days while under refrigeration the storage period could be extended upto 20 days. With regard to development of acidity (Table 1) in different samples under room temperature iron contained samples exhibited the highest values followed by brass tinned brass aluminium and stainless-steel.

TABLE 3

Showing the percentage acidity at different storage periods in khoa wrapped in different wrappers

Wrappers	Storage Periods at Room Temperature in Days (A)				
	1	3	5	7	10
Control	0 100	0 120	0 274	0 688	0 958
Sterilized butter paper	0 100	0 114	0 250	0 646	0 892
Sterilized butter paper soaked in sodium propionate solution.	0 100	0 126	0 342	0 840	1 240
Wrappers	**At Refrigeration Temperature in Days (B)**				
	1	7	15	20	25
Control	0 100	0 114	0 212	0 568	0 808
Sterilized butter paper	0 100	0 110	0 198	0 514	0 782
Sterilized butter paper soaked in sodium propionate solution.	0 100	0 142	0 274	0 686	0 920

A marked difference in acidity was noted after a storage period of 10 days both under room and refrigeration conditions. The rate of development of acidity remained almost the same in case of iron, but with other metals there was a decreasing trend after 7 days of storage At room temperature the final values observed were 1 360 1 000 0.976 0 926 0.853 per cent for iron brass, tinned brass, aluminium and stainless-steel respectively Under refrigeration the rate of acidity development was slow upto 15 days of storage followed by a subsequent rapid increase particularly in case of iron (1 09 per cent) The values for brass, tinned brass, aluminium and stainless-steel were 0 830 0 796 0 770 and 0 706 per cent respectively In acidity no significant effect of different wrapper treatments was visible upto five days

at room temperature and upto 15 days under refrigeration Thereafter (Table 3 Fig 3) an abrupt increase particularly in samples packed in deterged papers was noted (Table 3 and Fig 3) The final acidities reached were 1.240, 0.958 and 0.892 under room temperature and 0.920 0.808 and 0.782 per cent under refrigeration temperature with deterged paper ordinary butter paper and sterilized butter paper respectively The initial higher acidity percentages in case of deterged paper samples and their maintenance throughout the storage period both at room and refrigeration temperatures in comparison to control and sterilized wrappers, can be explained on the basis that such samples were less infested with mould mycelia which consume lot of acids than the other two.

Almost constant peroxide values (1.40-1.50) were observed in different samples with various treatments in the beginning of th analyses. There was a significant increase in these values during storage being faster at room temperature than under refrigeration. Samples prepared in iron *karahis* exhibited highest initial and final peroxide values in comparison to stainless-steel which gave the lowest values. The other metals used in these investigations showed an irregular trend during the intermediate stages of storage, but the values could not cross the upper and lower limits set by iron and stainless-steel respectively Regarding the effect of differently treated wrappers on peroxide value (Table 4) the samples in unsterilized wrappers gave the highest values and deterged wrappers the lowest with samples in sterilized wrappers falling in between the two extremes under both the storage conditions (Table 4 and Fig 4)

TABLE 4

Showing the peroxide value at different storage periods in Khoa wrapped in different wrappers

Wrappers	Storage Periods at Room Temperature in Days (A)				
	1	3	5	7	10
Control	1 46	5 30	7 94	13 84	17 39
Sterilized butter paper	1 46	5 30	7 78	13 20	16 83
Butter paper soaked in sodium propionate solution	1 46	5 14	7 12	11 84	15 80

Wrappers	Under Refrigeration Temperature in Days (B)				
	1	7	15	20	25
Control	1 46	3 06	5 56	8 04	10 46
Sterilized butter paper	1 46	2 88	5 06	7 80	10 24
Butter paper soaked in sodium propionate solution	1 46	2 44	4 06	7 48	10 02

A continuous and constant decrease in moisture content of *khoa* has been noted in the present study both under room temperature and refrigerated conditions irrespective of the metal used for its preparation With respect to different wrappers the fall in moisture content under room temperature was more in samples wrapped in unsterile papers than deterged and sterilized ones. No significant difference however could be noticed under the refrigeration conditions (Table 5)

TABLE 5

Showing the variations in moisture per cent of khoa wrapped in different wrappers

Wrappers	Storage Periods at Room Temperature In Days (A)				
	1	3	5	7	10
Control	32 00	27 71	24 34	20 83	18 07
Sterilized butter paper	32 00	28.52	24 62	21 78	19 75
Butter paper soaked in sodium propionate solution	32 00	28 14	25 20	21 95	19 25

Wrappers	At Refrigeration Temperature in Days (B)				
	1	7	15	20	23
Control	32 00	28 58	24 51	22 31	20 71
Sterilized butter paper	32 00	28 89	24 82	22 41	20 83
Butter Paper soaked in sodium propionate solution	32 00	29 49	23 43	22 26	20 84

SUMMARY

Khoa is one of the most important products of our dairy industry utilizing about 4.3 per cent of the total milk produced. The practices associated with its preparation such as type of pan type of wrappers, source of heat and the extent of concentration vary widely from place to place and in different seasons. The variation in the quality of *khoa* in the market poses some practical difficulties. To overcome this short-coming scanty research by some workers has been going on from time to time but the information available so far cannot be built up to recommend measures for any improvement in the quality of this product It was with the aim of strengthening the present available information that a few variables were studied. The results suggest that the greatest resistance to deterioration is offered by stainless-steel, next in order being aluminium, brass, tinned brass and iron

the life of the product being 6, 5 4 and 3 days in different cases respectively at room temperature. Under refrigeration life could be enhanced to 15-20 days, the lower figure being associated with iron and higher with the remaining metal pans.

The effect of different types of wrappers showed a better resistance towards mould growth than the control. Thus resistance was more in samples wrapped in paper treated with sodium propionate solution, followed by sterilized butter paper under both the storage conditions. The spoilage was set after 5th day at room temperature and 20 days under refrigeration. Deterged papers proved better than the sterilized and ordinary ones as indicated by acidity and peroxide values during different periods of storage It may be inferred, therefore, that *khoa* prepared in stainless-steel and wrapped in deterged paper confirms to the desired keeping quality for a longer duration of time particularly if stored at lower temperature.

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A continuous and constant decrease in moisture content of *khoa* has been noted in the present study both under room temperature and refrigerated conditions irrespective of the metal used for its preparation. With respect to different wrappers the fall in moisture content under room temperature was more in samples wrapped in unsterile papers than deterged and sterilized ones. No significant difference, however could be noticed under the refrigeration conditions (Table 5)

TABLE 5

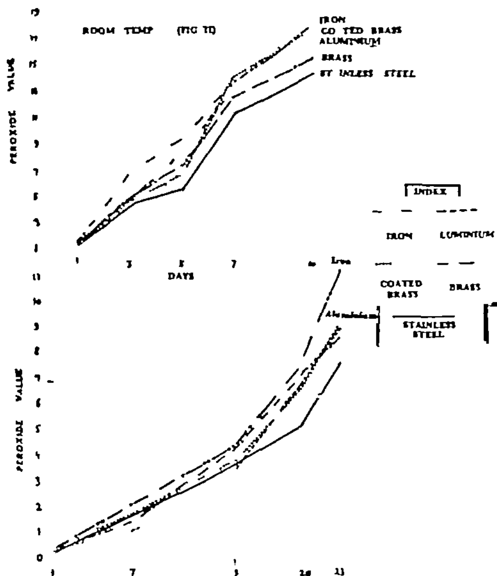
Showing the variations in moisture per cent of khoa wrapped in different wrappers

Wrappers	Storage Periods at Room Temperature in Days (A)				
	1	3	5	7	10
Control	32 00	27 71	24 34	20 83	18 07
Sterilized butter paper	32 00	28 52	24 62	21 78	19 75
Butter paper soaked in sodium propionate solution	32 00	28 14	23 20	21 93	19 25

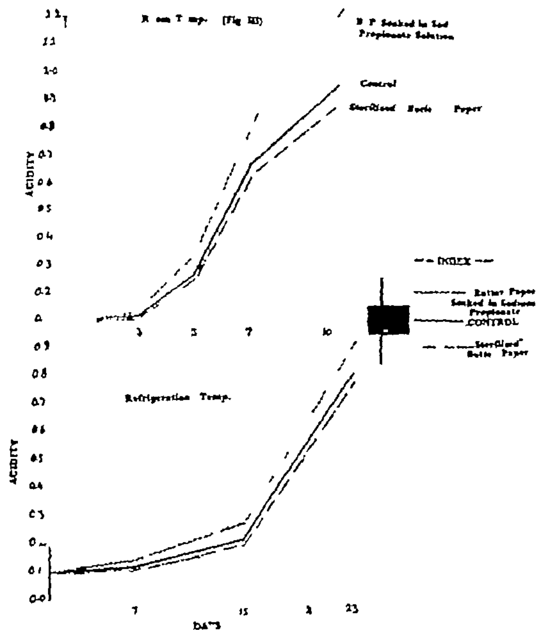
Wrappers	At Refrigeration Temperature in Days (B)				
	1	7	15	20	23
Control	32 00	28 58	24 51	22 31	20 71
Sterilized butter paper	32 00	28 89	24 82	22 41	20 83
Butter Paper soaked in sodium propionate solution	32 00	29 49	23 43	22 26	20 84

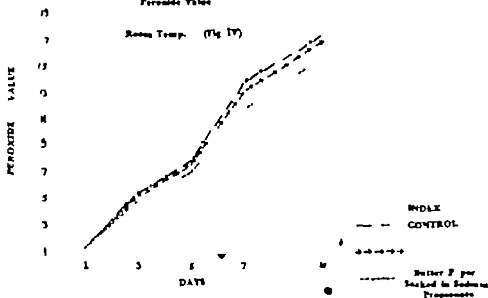
SUMMARY

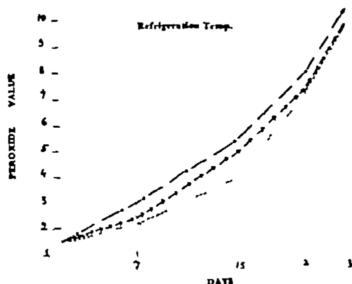
Khoa is one of the most important products of our dairy industry utilizing about 4.3 per cent of the total milk produced. The practices associated with its preparation such as type of pan type of wrappers, source of heat and the extent of concentration vary widely from place to place and in different seasons. The variation in the quality of *khoa* in the market poses some practical difficulties. To overcome this short-coming scanty research by some workers has been going on from time to time but the information available so far cannot be built up to recommend measures for any improvement in the quality of this product. It was with the aim of strengthening the present available information that a few variables were studied. The results suggest that the greatest resistance to deterioration is offered by stainless-steel next in order being aluminium, brass, tinned brass and iron


EFFECT OF METALS ON
PEROXIDE VALUE
ROOM TEMP
PEROXIDE VALUE
IRON
ALUMINIUM
BRASS
DAYS
INDEX
IRON
COATED BRASS
BRASS
STAINLESS STEEL
PEROXIDE VALUE

Showing the effect of wrappers on acidity




Effect of wrapper on
Peroxide Value
Room Temp. (Fig IV)
PEROXIDE VALUE
DAYS
INDEX
CONTROL
Butter P per
Soaked in Sodium
Propionate


Refrigeration Temp.
PEROXIDE VALUE
DAYS

ON A FILARIAL NEMATODE FROM DOMESTIC FOWL

DHARMENDRA NATH

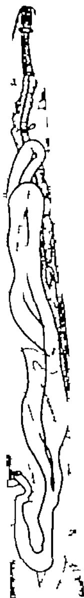
Department of Parasitology U.P College of Vet Sc & Animal Husbandry Mathura

During post mortem examinations of domestic fowl the heart, on one occasion yielded from one of it s ventricles two female specimens of a filarial nematode Subsequent search for more specimens including adult males proved futile. One of these specimens was studied alive and after fixation in hot 70 % alcohol was cleared in glycerine and later cut for serial sections. The parasite is herein briefly described

The worms, of creamy white colour with the attenuated anterior and posterior ends and the cuticle finely striated longitudinally measure 4.3-4 4 cm. in length. The breadth at head region is 0 104-0 106 mm., at the mid body 0.522-0.537 mm. and at anus 0 16-0 164 mm. The simple mouth has no lips but the head carries two lateral and four subventral papillae (Fig 2) The oesophagus is divisible into an anterior muscular and a posterior glandular part (Fig 1) of 0.368-0 492 mm. and 1.269-1 432 mm. in length and 0 074-0 075 mm and 0 160-0 164 mm in maximum width respectively The nerve ring and excretory pore lie at a distance of 0 2-0 23 mm. and 0 716 mm. respectively from the anterior end. The vagina, before opening at the vulva, forms a loop (Fig 4) which lies behind the oesophageo-intestinal junction at a distance of 3.25 mm from the anterior end. The anus is situated at a distance of 0 120-0 126 mm from the tip of the bluntly rounded tail (Fig 3) No papillae could be observed at this region.

These specimens resemble the material reported by Bhalerao and Rao (1944) who had studied a single female specimen recovered from the heart of a Black Minorca cock from Hyderabad which was described and assigned to a new species under the genus *Bhalfilaria* tentatively created for it s reception Skrjabin et. Shikhobalova (1948) in their comprehensive treatment of this group have not recognised its validity and accordingly include it amongst the in completely known forms. The joint authors description, however from a perusal of the foregoing account, would appear incomplete in respect of the following points (i) two lateral papillae on each side have been stated to be present at the posterior end (such papillae were neither observed nor the pair of large papillae, also mentioned to lie at a distance of 0 113 mm. infront of the anus, were noted) (ii) the vulva and vagina, not seen by Joint authors inspite of prolonged observation, are definitely present and distinct in their outline.

Bhalerao and Rao commenting on this form, appended remarks on certain species of other genera like *Splendidofilaria* Skrjabin, 1923 *Chandlerella* Yorke et Maplestones, 1926 *Macdonaldius* Khanna, 1933 *Parachocerca* Peters 1936 and *Cardiofilaria* Strom 1937 and from amongst these the specimen described was compared with *Macdonaldius* in which according to these authors, the position



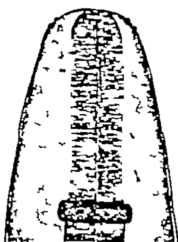


Fig. 2





Fig. 4

Fig. 1   Fig. 3

Fig 1 Anterior end of the female worm

Fig 2. Head region of (1) showing two lateral and four subventral papillae (magnified)

Fig 3. Posterior end of (1) showing absence of papillae at this end (magnified)

Fig 4 Vulvar region of (1) showing the well developed vagina forming a loop

GENETIC STUDIES ON FERTILITY HATCHABILITY AND CHICK VIABILITY IN WHITE LEGHORNS

BHANU PRATAP SINGH[1]
Department of Animal Husbandry U P., India.

From data[2] relating to 5,570 eggs of 196 single comb White Leghorn pullets, set at weekly intervals for 11 weeks mean fertility was found to be 90 16% mean hatchability of fertile eggs set 50.89 / and mortality among hatched out chicks to 6 weeks of age 28.01%

Cockerels gave 5 74% and 6.09% higher fertility and hatchability than cocks. Viability upto 6 weeks after hatching was significantly (P/_0.05) higher from cock matings than from cockerel matings This indicates that progeny from cock matings, if used for further breeding would improve the longivity of the subsequent generations.

Strain crosses gave lower fertility but higher embryonic and post embryonic viability than pure strain matings. But none of the results were significant. For every 3 % increase in inbreeding chick mortality was expected to increase 2.17% in 6 weeks.

An increase in 5 more eggs set per pullets gave 0.32% and 2.88% rise in fertility and hatchability of fertile eggs.

From the hatching results of this investigation it was found out that eggs weighing upto 56 gms. from birds which normally lay eggs of uniform size in clutches of 3 having shape index 72 75.9 and evaporation loss 7 11.5% on 14th day of incubation give the best hatching results.

Viability of chicks hatching from eggs weighing upto 56 gms. and representing over 65% of the original egg content on hatching was much superior to others. Viability of chicks which hatched normally but after usual incubation period of 21 days were significantly lower

About 14% of embryos died during 6-14 days of incubation and 70% during 18-24 days. 30% of the overall embryonic deaths were due to various malpositions which represented 10% of fertile eggs.

Heritability estimates on arc-sine /proportion scale for fertility hatchability of fertile eggs, embryonic and post embryonic viability by full-sib correlation method were 23.8% 3 7% 12 8 % and 53 8 / respectively

Selection of pullet families with higher maternal effects and cockerels for better general combining ability promises rapid improvement of fertility

1 Present address Department of Animal Husbandry University of Minnesota U.S.A.

2. The data was collected during 1960-61 t State Poultry Farm Mathura

Specific combining ability was found to be more important for embryonic viability

According to this investigation maximum genetic gains can be expected by testing a minimum of 10 pullets per dam and 25 eggs per pullet for hatch per cent of total eggs set.

High genetic correlation of fertility with hatchability embryonic and post embryonic viability indicates that more emphasis should be placed on fertility of eggs for genetic improvement of all other traits.

On the basis of above findings the indices giving relatively optimum economic emphasis on concerned characters are

I_1 = 3 72 Fertility + Hatch per cent of fertile eggs.

I_2 = 1 92 Fertility + 1.53 Embryonic viability of fertile eggs + Post embryonic viability (Upto 3 weeks)

STUDIES ON THE BIOLOGY OF THE GRAYLING *THYMALLUS THYMALLUS* L. TOGETHER WITH AN INVESTIGATION OF THE MINUTE ANATOMY OF ITS ALIMENTARY CANAL

CHANDRA PAL SINGH
Lecturer in Zoology, Agra College, Agra.

1 The distribution of grayling is dealt with especial reference to Britain

2. The physico-chemical conditions (temperature pH, calcium chloride and oxygen contents) of the Hampshire River Avon at Great Durnford are recorded

3 The relative abundance of the fauna of the Avon at Great Durnford is assessed and seasonal changes in it discussed.

4 The food of 305 grayling caught at different times of the year from the Avon and its tributaries is studied by analysis of the gut contents The food consisted of a great variety of animals, mainly from the river bed

5 The feeding habits of grayling under experimental conditions have been studied. Gut analysis and experimental results both suggest a correlation between different size, kind and proportions of food organisms eaten by grayling of different size

6 Easily accessible and more actively moving animals are more readily eaten by grayling

7 No seasonal fasting occurs in grayling, but feeding slows down after mid-summer It is suggested that the feeding of grayling is reduced at higher temperatures.

8. Of the structures investigated the scales of grayling were found to be most satisfactory for the study of growth and age.

9 Growth is rapid during the first and second year age group, after which it slows down.

10 There was no statistical difference between the lengths attained by male and female grayling of comparable age.

11 Generally males reach maturity earlier (1 year age group) than females (2 year age group)

12 No spawning marks occur on grayling scales. It is suggested that this may be correlated with continuous feeding by the fish throughout the spawning period.

13 Narrow rings are formed on the scales in July August and probably in September The period of rapid growth in grayling is from March—June—July

14 Grayling spawn in March/April The time taken by grayling eggs to hatch varies from 18 to 25 days according to temperature.

15 The condition factor Kn was investigated for male and female grayling

16 *Echinorhynchus truttae* was found as a parasite in the posterior part of the alimentary canal of grayling

17 An attempt has also been made to correlate the growth and distribution of grayling with the factors of environment. On the whole grayling appears to be a non-exacting' species.

18 Seasonal variations in the temperature of the river water appear largely to affect the physiology of the fish through basal metabolism, maintainance requirement, relation condition factor conversion and absorption of food etc.

19 Grayling has been found to have a lower degree of optimal temperature requirements in comparison to trout.

20 The alimentary canal of grayling can be distinguished into mouth buccal cavity pharynx, oesophagus, stomach, intestinal caecae, and intestine.

21 The oral orifice is moderate in size and ventral.

22. Small conical teeth are present on the upper jaw A tongue is present. The pharyngeal teeth are present on two small oval pads

23 There is no distinct demarcation between the oesophagus and the stomach. The stomach is distinguished into the cardiac, cardio-pyloric and pyloric regions.

24 The pyloric valve is present in the form of extreme constriction of the muscularis at the posterior end of the pyloric region of stomach

25 Numerous pyloric caecae are present.

26. The intestine is almost uniform in its histological structure throughout and there is no demarcation between the intestine and the rectum.

27 Taste-buds are present on the labial palps, tongue, buccal cavity pharynx and the anterior-most portion of the oesophagus. Their number decreases gradually from the labial palp to the oesophagus.

28. The stratum compactum is absent in the stomach, while it is distinct in the intestine

29 The mucus-secreting cells increase from the anterior portion of the buccal cavity to the oesophagus. They are also present in the intestine.

30 Four distinct layers form the wall of the oesophagus stomach and intestine.

A STUDY ON THE PHYSIOLOGY OF DIGESTION IN *COLISA FASCIATA* (BLOCH & SCHNEIDER)

V P AGRAWAL AND RAJ VIR SINGH
Zoology Department, D A V College Muzaffarnagar

Contents

INTRODUCTION

During the Collection of the local fish fauna of Muzaffarnagar district, it was found that the fish, *Colisa fasciata* (Bloch & Schneider) also known as *Trichogaster fasciatus* is of very common occurence. The fish is of very wide distribution and is abundantly available in North East India, Burma and Malaya

The fish, collected from the rivers, streams and small ponds of the locality could be easily reared in the aquarium and survived under artifical conditions, for months together without any extra precautions.

By the study of the gut contents of the freshly collected fish, it was found that the fish mainly feeds on large algal filaments, but the starved fish could also be fed on animal plankton.

The mouth of *Colisa* as described by authors (1961) is a small aperture situated on the dorsal side of the snout the fish occasionally comes on the surface to engulf atmospheric air The rest of the alimentary canal consists of the buccal cavity pharynx, oesophagus, the cardiac stomach, pyloric stomach, long and very much coiled intestine, which has a pair of caeca at its junction with the stomach. The intestine insensibly passes into the small rectum.

The liver is a large brownish mass, covering the oesophagus and the stomach the gall bladder is a conspicuous bag, dark green in colour

HISTORICAL REVIEW

The morphology and histology of alimentary canal of the teleostean fishes has been studied by many workers such as Blake (1930 & 1936) Rogick (1931) Sarbahi (1939) Curry (1939) etc. The investigations on the physio-

logy of digestion in fishes have been very few Workers on the food of fishes have made only incidental observations on the physiology of digestion Rahimullah (1945) instituted a comparison between the physiology of pyloric caeca in the carnivorous fish *Ophiocephalus striatus* and the herbivorous fish, *Osphronemus goramy* Wigglesworth (1927) has given a very good account of the process of digestion in cockroach.

A review of the work done on the physiology of digestion in fishes is given by Brown (1957) Sullivan (1907) worked on the physiology of digestion in a number of elasmobranch fishes.

Material and Methods

The fishes for this work were mainly collected from "Kali Nadi" which runs through Muzaffarnagar The fishes, for experiments, were kept in aquarium under different conditions. To determine the hydrogen ion-concentration of the normal feeding fishes, the alive animals were immediately dissected after their collection and the alimentary canal together with the associated glands were removed. The different parts of the gut were carefully separated and thoroughly washed in distilled water Both capillary indicator and indicator paper methods were used to determine the pH in the different parts of the gut.

A few fishes were starved by keeping them in distilled water for about 80 hours so as to clear the alimentary canal, and the pH in the different parts of the gut was noted as above. Finally a few fishes were first starved for about 80 hours and then fed for about 8 hours on specific diets the pH in different parts of the gut was again calculated.

For the qualitative analysis of the enzymes in the different parts of the gut and the digestive glands, the extracts of the different parts of the alimentary canal were prepared. The living fishes were first starved for about a week, to clear off the gut, and were then dissected in distilled water The different parts of the alimentary canal were separated off and were ground up with a little thymol and a few drops of glycerol to form a thick emulsion This was diluted to 10 per cent solution by 50 per cent glycerine. The solution after being centrifuged, and decanted was kept in a small tube the rest of th tube was filled with toluene The extracts were allowed to stand for about 72 hours, before they were tested for different enzymes.

pH Determination

The determination of pH in the different parts of the digestive tract, is an important aspect of the study of the process of digestion, for different enzymes act optimally under different ion-concentrations.

The pH of the freshly collected animals was measured as described above. The readings of the six fishes were set out in Table I and the average in each case was calculated as given in the table

TABLE 1

Showing pH in the normal feeding fish

S. No.	Oesophagus pH	Cardiac stomach pH	Pyloric stomach pH	Liver pH	Gall bladder pH	Intestine pH	Caeca pH
1	5 6	5 7	5 6	6 3	6 5	5 8	5 8
	5 5	5 6	5 7	6 2	6 6	5 7	5 7
3	5 6	5 8	5 7	6 4	6 5	5 8	5 8
4	5 6	5 7	5 7	6 3	6 5	5 9	5 8
5	5 6	5 7	5 7	6 3	6 5	5 8	5 8
Average pH	5 6	5 7	5 7	6 3	6 5	5 8	5 8

The above table shows that, there is no marked difference of pH in the different parts of the gut. The oesophagus, cardiac stomach and pyloric stomach are more acidic, while the medium in the intestine, liver and gall bladder is nearer neutrality

Brown (1957) has given that the acidity in the stomach of the Teleostei is much less marked than in elasmobranchs. According to him the average pH in the stomach is 5 6 while that of the bile and gall bladder in the vicinity of 6 4 and 7.2 Al Hussaini (1949b) found that pH of intestine in *Rutilus Gobio* and *Cyprinus* was from 6 12 to 7 72 This shows that the pH in the different parts of th gut of *Colisa* as recorded here is almost the same as given by other authors.

A few fishes were starved for about 80 hours by keeping them in filtered water the pH in the different parts of the gut of these fishes was also tested as before. The result of these readings is set out in Table 2.

TABLE 2

Showing pH in the gut of starved fishes

S. No.	Oesophagus pH	Cardiac stomach pH	Pyloric stomach pH	Liver pH	Intestine pH	Caeca pH	Rectum pH
1	6 1	5 9	6 5	6 5	6 8	6 8	6 9
2	6 0	6 0	6 5	6 4	6 9	6 8	7 0
3	6 1	5 9	6 5	6 5	7 0	6 9	7 0
Average pH	6 1	5 9	6 5	6 5	6 9	6 8	7 0

The above table shows that the pH in the gut of starved fishes become less acidic than in the normal feeding fishes This change is more pronounced in the pyloric stomach and intestine. This difference may be due to the fact that the pH in the normal fishes is interfered by the presence of food in the gut while in the starved fishes it is pH only of the internal secretion with in the lumen of the gut.

It implies, therefore, that the pH in the pyloric stomach intestine and caecum, where the process of digestion is active is more or less towards neutrality

A few fishes were then, starved for about 50 hours and then fed on selective diet such as animal plankton for about 8 hours the pH in the different parts of the gut in these animals was as set out in Table 3

TABLE 3

Showing pH in the gut of fishes fed on selected diet.

S N	Oesophagus pH	Cardiac stomach pH	Pyloric stomach pH	Liver pH	Intestine pH	Caecum pH	Rectum pH
1	5 8	5 8	6 2	6 5	6 5	6 6	6 9
2	5 7	5 9	6 3	6 6	6 6	6 7	6 9
3	5 8	5 8	6 2	6 5	6 5	6 6	7 0
Average pH	5 8	5 8	6 2	6 5	6 5	6 6	6 9

When all these three tables are compared it is found that the selective feeding only slightly alters the medium in the different parts of the gut especially with respect to starved fishes.

Qualitative Estimation of Enzymes in the Different Parts of the Gut in Colisa Fasciata

Yonge (1954) has stated that there is a definite correlation between the food of any animal and the nature and relative strength of its digestive enzymes.

The *following experiments were performed in order to investigate* as to where the digestive enzymes are secreted. The extracts were prepared as described before and were tested for various enzymes with respect to carbohydrates, proteins and fats.

The following experiments were performed in order to investigate the different enzymes

Amylase—In order to investigate the presence or absence of amylase a few drops of the tissue suspension from the different regions of the gut, were incubated with a few drops of 0 5 % boiled starch solution and the rest of the *tube in each case, was filled with toluene. Another set of tubes with a few* drops of boiled extracts and a few drops of starch solution also filled with toluene, acted as controls. The incubated solutions were tested for amylase, after different intervals with the iodine solution, it was found that the colour of the iodine remained unchanged in the cases where the starch had been hydrolysed, while in all the control experiments and some others where the starch was not digested, the colour of the iodine had changed to blue. The potassium-iodide-iodine test also *confirmed the results.*

The incubated solutions with positive results were further tested for maltose, which is the first product of the hydrolysis of starch, by the picramic acid test. Four drops of the *incubated solutions, one drop of 10% sodium-*hydroxide solution and two drops of saturated aquous solution of picric acid were kept for about 15 minutes in an electric oven at 60° C. the yellow colour of the picric acid had changed to reddish brown colour of picramic acid, wherever the starch had hydrolised For further confirmation, Fehling's test and Benedict's tests were also performed, which gave the results as set out in the different tables

Maltase—A 2% maltose solution was similarly incubated with a few drops of the different extracts from the different parts of the alimentary canal of the fish. After 24 hours a portion of the mixture was tested for glucose by the osazone test. A few drops of the *incubated mixture* ... few ... of phenyl-hydrazine-reagent were kept in the oven for al[illegible] an

100° C. the yellow crystals formed in the mixture, were examined under the microscope to see if the needle-shaped crystals of glucose-osazone are present.

To confirm the presence of maltase, Barfoed's test for monosaccaride was performed A small quantity of acetic acid was added to a solution of copper acetate, which is used to restrain the action of disaccharide, a reddish-brown precipitate appeared, where the maltose had been digested.

Lactase—A 2 % solution of lactose was also incubated with a few drops of the extracts from the different parts of th gut the control experiments were also set out in all these cases. The digestion of the lactose into glucose was tested by osazone and Barfoed's tests. The results are set out in different tables given in the following pages.

Invertase—A few drops of 5% sucrose solution were incubated with a few drops of different extracts. The incubated solutions were tested by Benedict's and Fehling's tests, the results are given in the different tables.

Glycogenase—A few drops of the saturated solution of glycogen were also incubated with a few drops of the different extracts the solutions were tested for the presence of any reducing sugars by the Benedict's and Fehling's tests.

Raffinase—A 1% solution of raffinose when similarly incubated with different extracts, and tested for glucose, gave in almost all cases, the negative results.

Inulinase—Similar experiments were performed with respect to a 1% solution of inulin and the results are tabulated in the following pages.

Salicinase—A 1% salicin solution was also incubated with the extracts and the results are tabulated.

Lipase—The presence of lipase was investigated by experimentation on condensed milk. Two drops of bromo-thymol blue were added to 25 cc of a 10% solution of the condensed milk. To this solution was added 1% sodium hydroxide solution until the mixture turned light blue in colour In each case, 1 cc. of blue milk solution and a few drops of the different extracts were incubated in a tube, the rest of the tube was filled with toluene. After a few hours of incubation the colour of the milk, wherever the fat had been digested, had changed to yellow The control experiments on the other hand, did not show any change in the colour of the milk

To confirm these observations, another test was performed with olive oil 10 drops of olive oil were dissolved in 4 cc. of absolute alcohol by thorough shaking 4 cc. of hot water were added to this mixture To this mixture, when it had cooled, 10 drops of phenol-red were added. A few drops of 0.01 N NaoH were added to make the emulsion faintly pink. 2 cc. of this mixture were incubated with 1 cc. of the different extracts. It was observed that after a few minutes the pink colour of the mixture had changed to yellow confirming that the fat has been digested in these cases.

Proteases—The digestion of proteins was tested by incubating 1 cc. of 10% gelatine with 1 cc. of the different extracts. The gelatine in thos

cases where proteases are present, gets liquified after about 24 hours in the control experiments, on the other hand, the gelatine remained in the solid state

To summarise these results, the following tables are given with respect to the extracts from the different parts of the gut. In all instances the pH of the extract was noted before and after the reaction, which remained almost unchanged.

The sign ++ as given in the tables, denote a vigorous reaction sign + indicates a definite positive reaction ± means traces of reaction while the sign — means no reaction at all.

TABLE 4

Showing the presence or absence of the enzymes in the liver of the fish.

Substrate	Duration of reaction and extent of digestion			Control experiments After 72 hours
	After 24 hours	After 48 hours	After 72 hours	
1% Starch solution	++	++	++	—
Saturated solution of Glycogen	±	+	+	—
5% Sucrose solution	+	++	++	—
2% Maltose solution	—	—	—	—
2% Lactose solution	—	—	—	—
1% Raffinose solution	+	++	++	—
1% Inulin solution	+	++	++	—
1% Salicin solution	+	++	++	—
1% Gelatine solution	Gelatine is liquified after about 30 hours			Remained solid
Condensed milk etc.	Colour changed to yellow after 15 minutes			Colour unchanged

The Table 4 shows that most of the carbohydrates are digested by the enzymes secreted by the liver-cells of the fish. However maltase and lactase are completely absent The proteases, though not very strong are also present.

The extract of the pyloric caeca from a few fishes was also prepared and the experiments with the different substrates were set up as before The results after different intervals are tabulated in Table 5

TABLE 5

Showing the presence or absence of the different enzymes in the pyloric caeca

Substrate	Duration of reaction and extent of digestion			Control experiments After 72 hours
	After 24 hours	After 48 hours	After 72 hours	
1% Starch solution	—	—	—	—
Saturated solution of Glycogen	+	+	+	—
5% Sucrose solution	+	+	+	—
2% Maltose solution	—	—	—	—
2% Lactose solution	—	—	—	—
1% Raffinose solution	—	—	—	—
1% Inulin solution	—	—	—	—
1% Salicin solution	—	—	—	—
10% Gelatine solution	Remained solid			Remained solid
Condensed milk etc	Colour remained unchanged			Remained unchanged

The Table 5 shows that caeca take but little part in the digestion of food. Among carbohydrate hyrolysing enzymes, only weak glycogenase and sucrase are present the proteases and lipase are also absent

Similar experiments were set up with the extract of the stomach of the fish and the results are recorded in Table 6

TABLE 6

Showing the presence or absence of the different enzymes in the stomach.

Substrate	Duration of reaction and extent of digestion			Control experiments After 72 hours
	After 24 hours	After 48 hours	After 72 hours	
1% Starch solution	—	—	—	—
Saturated solution of Glycogen	±	±	±	—
5% Sucrose solution	—	—	—	—
2% Maltose solution	—	—	—	—
2% Lactose solution	—	—	—	—
1% Raffinose solution	—	—	—	—
1% Inulin solution	—	—	—	—
1% Salicin solution	—	—	—	—
10% Gelatine solution	Remained solid			Remained solid
Condensed milk etc	Colour remained unchanged			Remained unchanged

The above experiments show that no digestive enzymes are secreted either in the cardiac or in the pyloric stomach.

Similar experiments with the extract of the intestine showed that no enzymes are secreted in this part of the alimentary canal either

The above experiments on the determination of the hydrogen-ion-concentration and the qualitative estimation of enzymes in the different parts of the alimentary canal of *Colisa* reveal certain intersting facts.

The medium in the different parts of the gut is either neutral or weak acidic no where it is either strongly acidic or alkaline According to Baylis (1935) the acidity in the stomach of teleosts is much less, the average mean being 5·6. Almost similar results are found by us in the stomach of *Colisa*. The pH in the intestine of this fish also agrees with the results, as found by Al-Hussaini (1949b) in *Cyprinus*

The starvation of the fish slightly alters the pH in the different parts of the alimentary canal which shows that the starvation of the fish, stimulates the digestive cells to secrete more actively

The experiments on the qualitative estimation of enzymes show that the liver is the main organ, where most of the digestive enzymes are secreted

SUMMARY

1 *Colisa fasciata* is a mud feeding herbivorous fish, feeding mainly on algal filaments.

2. The fish has a small superior mouth, which leads into the buccal cavity The rest of the alimentary canal consists of the pharynx, the oesophagus, cardiac stomach, pyloric stomach, very long and coiled intestine and a small rectum the large unlobed liver in its natural position, almost completely covers the oesophagus and the stomach.

3. The hydrogen-ion-concentration in the different parts of the gut differs only slightly with each other it is only weakly acid, being about 5·6 in the stomach and about 6.7 in the intestine.

4 It has been observed that the different enzymes are secreted by the liver-cells.

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STUDIES ON CHANGES IN CARBOHYDRATES DURING HUMIFICATION OF EUPHORBIA HIRTA (DHUDIA) LEAVES

MAN MOHAN NARAIN TANDON AND NEWTON RAM

Chemical Laboratories Agra College Agra.

ABSTRACT

The percentage of reducing and non-reducing sugars pectic substances, hemicelluloses non-cellulose polysaccharides and cellulose constituents of non-leguminous Euphorbia hirta (Dhudia) leaves humified alone and with soil at 15% moisture level extracted with different solvents have been estimated in each extract and at different specific periods. Results show that decomposition of carbohydrates is much greater in T S (Leaves mixed with soil 1.20) as compared to O M (Humified leaves only) This emphasizes the role of micro-organisms which develop in the soil during humification. Decomposition of sugars proceeds with the decomposition of non-reducing to reducing sugars followed by oxidation In the case of treated soil (Leaves mixed with soil 1.20) there is an increase after 60 days of humification, in reducing as well as non-reducing sugars, which suggest the synthesis of sugars by carbon fixing bacteria

The order of decomposition of these carbohydrates during humification of organic matter is as follows :

Cellulose > Non-reducing sugars > Pectic substances > Reducing sugars > Non-cellulose polysaccharides > Hemicelluloses > Lignin.

The order of decomposition in treated soil is as follows :

Pectic substances > Cellulose > Non-cellulose Polysaccharides > Non reducing sugars > Reducing sugars > Hemicelluloses.

From the data of the organic carbon total nitrogen, mineral nitrogen and C.N ratio of the samples it appears that C.N ratio decreases in all cases. The decrease is maximum in leaves when humified alone. From the increase in ammoniacal and nitrate nitrogen and greater decomposition of carbohydrates in T S it has been suggested that Euphorbia hirta (Dhudia) an abundantly found non-leguminous weed may increase the soil fertility if used as a potential green manure

INTRODUCTION

The soil organic matter is mainly derived by the decomposition of plant and animal residues, by the action of soil micro-organisms. The activity of soil micro-organisms leads to two main products—Nitrogen (as nitrate and ammoniacal nitrogen) and Carbon dioxide—which are essential for the growth of

higher plants. The cell contents of the plants are rich in proteins and sugars while the rest of the structural material contains high polymers of sugars, fats and proteins. A I Schtshukina[1] observed that the addition of aqueous extract of straw containing pectins and sugar etc., increases the size of soil aggregates thus leading to better plant growth. James P Martin[7] also says that polysacchandes bring about aggregation of the soil. Carbohydrates on decomposition, aerobically and anaerobically form carbon dioxide through the intermediate formation of organic acids like maleic acid, fumaric acid, lactic acid and oxalic acid etc.

The importance of carbon dioxide for the soil and growing plants can be judged by the work of Lundegardh[3] and others—who reported that the carbon dioxide liberated by decomposition of soil organic matter tends to counterbalance the deficit in normal atmospheric concentration created by the assimilation of atmospheric carbon dioxide by the growing plants in the field. Meyer and Anderson[3] have reported that the carbon dioxide concentration in the atmosphere is the factor most frequently limiting the photosynthesis of well lighted plant tissues. D Fehr[4] observed that carbon dioxide content of the air in wood is conditioned by the simultaneous respiration of soil and is considerably influenced by the acidity of the soil A Rippel and H. Bortels[5] said that Aspergillus niger develops poorly in an atmosphere deprived of carbon dioxide and it is concluded that carbon dioxide is itself necessary for the plant cell. G E Rockwel and J H. High Beyer[6] said that Bacteria, yeasts and moulds require carbon dioxide as a source of carbon. M. S. Raju[8] observed that carbon dioxide production was increased by the addition of carbohydrates starch cellulose and glucose. Maria S Calabadi[9] said that an aerobic attack of bacteria on cellulose produces a colloidal substance which improves physical properties thus making it more resistant towards soil erosion Richard and Norman[10] suggested that leguminous plants decomposed more rapidly because of their higher nitrogen content and that a specified amount of nitrogen was necessary for rapid decomposition. Liesche[11] and Daji[12] observed that a balanced proportion of the available nitrogen and carbohydrate compounds in the plant materials controls its decomposition With this aim in view we have studied the decomposition of all carbohydrates in the leaves of Euphorbia hirta on humification and its effect on the fertility of the soil.

Experimental

For our experiments the weed Euphorbia hirta was collected, and its adhering soil was removed. The plant was divided into three portions i e., leaf stem and root. All portions were dried in shade. Then they were powdered and sieved through 60 mm mesh The soil was collected from a cultivated land. Several pits were dig at the distance of one yard each and from each pit soil was taken after 8 inches of depth. Soil collected from these pits was mixed, dried in shade and sieved through 100 m. m mesh.

Finely powdered leaves (10 g) of Euphorbia hirta were successively extracted with 70-80% hot ethanol, 4% cold NaOH, 17.5% NaOH and 72% H_2SO_4. Each extract was made to a known volume. The reducing sugars, total sugars and pectic substances in 70-80% hot ethanolic solution were estimated by lead clearing method as recommended by Loomis and Shull[12] and estimations were done by Benedicts solution (Haas and Hill[13]). Residue after extraction with 70-80% hot ethanol was treated with 200 cc of 4% NaOH (James Bonner[14]) and shaken in a mechanical shaker for two hours. This extract containing hemicellulose was filtered and divided into two portions, one portion was neutralised by adding a little excess of HCl and precipitate was filtered, washed and weighed as hemicellulose and the other portion was hydrolysed by 5% HCl nearly at 100°C for 3-4 hours. The excess of the acid was neutralised by adding a little excess of Na_2CO_3 before titrating with Benedicts solution. The residue obtained after treatment with 4% NaOH was extracted with 17.5% NaOH by shaking in a mechanical shaker for 2 hours. The extract was filtered and divided into two equal portions, one portion was neutralised with little excess of HCl and filtered. The precipitate so obtained was dried and weighed as non-cellulose polysaccharides. The other portion was hydrolysed with 5% HCl nearly at 100°C for 3-4 hours. The excess of acid was neutralised by adding a little excess of Na_2CO_3 before titrating with Benedicts solution. The residue obtained after treatment with 17.5% NaOH was treated with so much 72% H_2SO_4 that would cover the residue. After one hour this acid was diluted with distilled water and allowed to remain as such for two days. Then this was refluxed for 3-4 hours, cooled and filtered. The filtrate which contained hydrolysed cellulose was neutralised with excess of Na_2CO_3 and filtered. After filtration it was treated with animal charcoal and titrated with Benedicts solution. The residue obtained after 72% H_2SO_4 treatment was washed thoroughly with distilled water till it was free from acid and weighed as lignin.

Humification of the leaves alone and mixed with soil was done by technique of Crowther and Mirchandani[16] and Daji[15] for decomposition of plant materials. In our experiments 600 g of finely powdered leaves were mixed with 90 g of distilled water and total amount was maintained at 690 g for a fortnight by adding the required amount of water on alternate days. After a fortnight 60 g of the organic matter (Leaves alone) under humification study was taken out, and dried in shade and weighed. By taking the difference of weight of O. M. before and after drying and deducting the weight of water contained in 60 g of organic matter decomposition of organic matter during this period was determined. Now after first fortnight the weight of O. M. under humification was maintained at 630 g for a second fortnight. Second sample was taken out, dried and weighed and the decomposition for every fortnight was determined. At the end of 105 days it was observed that decomposition of leaves was 40% under humid condition.

TABLE 1

Changes in percentage of carbohydrates in organic matter

Time in No of days	Sugars 70-80% ethanol			Pectic substances	Hemicelluloses 4% NaOH	Non-cellulose polysaccharides 17 5% NaOH	Cellulose 72% H_2SO_4	Total carbohydrates excluding lignin	Lignin
	Reducing	Non-reducing	Total Sugars						
0	36	1 52	1 88	2 547	4 68	1 53	4 851	15 488	11 5
15	783	410	1 194	1 59	4 907	4 06	1 91	14 661	11 2
30	68	323	1 003	1.285	6 75	2 227	2 907	14 172	10 8
45	466	26	726	1 14	6 82	1 955	2 615	13 256	10 6
60	32	212	532	1 012	5 92	1 738	2 012	11 214	10 3
75	283	179	462	925	7 38	1 59	49	10 847	10 2
90	238	158	396	841	5 224	1 39	339	8 188	10 18
105	1836	116	30	745	3 1886	0508	2004	5 28	10 14

TABLE 2

Changes in percentage of carbohydrates in treated soil.

Time in No of days	Sugars 70-80% ethanol			Pectic Substances	Hemicelluloses 4% NaOH	Non-cellulose polysaccharides 17 5% NaOH	Cellulose 72% H_2SO_4	Total carbohydrates
	Reducing	Non-reducing	Total Sugars					
0	016	0774	0934	131	2684	689	256	8353
15	032	031	063	028	3373	061	245	7343
30	0308	0162	047	019	1584	112	22	5564
45	017	014	031	0083	10	000	4	5201
60	023	014	037	005	12	153	19	505
75	031	015	046	0022	1105	178	168	5017
90	033	016	049	0016	074	214	140	4868
105	0043	0152	0195	0015	1158	00904	0134	1595

TABLE I

Changes in percentage of carbohydrates in organic matter

Time in No of days	Sugars 70-80% ethanol			Pectic substances	Hemicelluloses 4% NaOH	Non-cellulose polysaccharides 17 5% NaOH	Cellulose 72% H_2SO_4	Total carbohydrates excluding lignin	Lignin
	Reducing	Non-reducing	Total Sugars						
0	30	1 52	1 88	2 547	4 60	1 53	4 851	15 488	11 5
15	783	410	1 194	[illegible]	4 907	4 00	1 91	14 601	11 2
30	60	323	1 005	1 285	0 75	2 227	2 907	14 172	10 8
45	406	20	720	1 14	6 82	1 955	2 615	13 250	10 6
60	32	212	532	1 012	5 02	1 758	2 012	11 214	10 3
75	283	179	402	925	7 38	1 59	47	10 617	10 2
90	238	158	300	811	5 224	1 59	339	8 188	10 18
105	1830	116	30	745	5 1890	8508	2001	5 28	10 14

TABLE 4

Percentage fixation and decomposition in total nitrogen nitrate nitrogen organic nitrogen mineral nitrogen organic carbon in organic matter treated soil and soil

Time in No. of Days	Organic ca bon			Total nitrogen			Nitrate nitrogen			Ammonical nitrogen			Organic nitrogen			Mineral nitrogen		
	O M	T.S.	S.	O M	T S.	S.	O M.	T.S.	S.	O.M	T S.	S.	O.M.	T.S.	S	O M.	T S	S
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	98 (−)	3 79 (−)	7 58 (−)	8 58 (−)	17 23 (−)	7 93 (−)	6 91 (+)	10 07 (+)	46 18 (−)	77 49 (−)	69 55 (−)	55 77 (−)	37 85 (+)	33 (−)	87 (−)	42 54 ( )	37 37 (−)	51 08 (−)
30	21 04 (−)	14 46 (−)	10 11 (−)	23 28 (−)	20 5 (−)	8 57 (−)	61 7 (−)	8 7 (−)	49 3 (−)	88 2 (−)	63 4 (−)	48 07 (−)	12 36 (+)	25 01 (−)	2 15 (−)	84 42 (−)	11 12 (−)	18 45 (−)
45	32 81 (−)	15 72 (−)	9 55 (−)	4 9 (+)	9 1 (+)	28 7 (−)	71 5 (−)	26 7 (−)	39 7 (−)	12 8 (−)	20 99 (−)	34 01 (−)	39 21 (+)	12 3 (+)	26 3 (−)	34 07 (−)	23 28 (−)	43 42 (−)
60	36 39 (−)	13 83 (−)	8 42 (−)	6 21 (−)	9 6 (+)	9 4 (+)	30 7 (−)	20 8 (−)	28 1 (−)	29 5 (+)	30 4 (−)	65 37 (−)	33 35 (+)	8 21 (+)	10 5 (+)	3 71 (+)	13 63 (+)	49 39 (−)
75	39 75 (−)	13 33 (−)	2 88 (−)	0 28 (+)	4 8 (+)	12 7 (+)	30 7 (−)	25 5 (−)	21 1 (−)	193 7 (+)	139 2 (+)	57 73 (+)	51 83 (+)	2 2 (−)	10 77 (+)	99 3 (+)	71 42 (+)	71 02 (+)
90	42 44 (−)	13 06 (−)	3 93 (−)	27 3 (+)	10 9 (+)	28 6 (+)	54 1 (−)	257 9 (+)	18 7 (−)	138 4 (+)	106 8 (+)	82 69 (+)	86 6 (+)	5 01 (−)	26 58 (+)	49 9 (+)	160 27 (+)	4 81 (+)
105	49 24 (−)	27 04 (−)	1 7 (−)	27 9 (+)	13 8 (+)	31 7 (+)	51 08 (−)	261 3 (+)	12 11 (−)	101 63 (+)	92 64 (+)	67 69 (+)	92 08 (+)	1 52 (−)	31 19 (+)	41 4 (+)	150 07 (+)	35 16 (+)

TABLE 5

Percentage fixation and decomposition of carbohydrates in organic matter and treated soil

Time in No. of days	Reducing sugars		Non-reducing sugars		Pectic Substances		Hemicelluloses		Non-cellulose Polysaccharides		Cellulose		Carbohydrates	
	O M	T S	O M	T S	O M	T S	O M	T S	O M	T S	O M	T S	O M	T S
15	117.5 (+)	100 (+)	73.09 (−)	57.18 (−)	37.57 (−)	69.61 (−)	1.85 (+)	25.07 (+)	165.30 (+)	31.46 (−)	60.02 (−)	4.29 (−)	6.63 (−)	12.09 (−)
30	91.6 (+)	90.25 (+)	78.71 (−)	77.42 (−)	49.01 (−)	85.82 (−)	41.23 (+)	40.98 (−)	14.21 (+)	25.81 (+)	40.47 (−)	4.08 (−)	8.49 (−)	21.41 (−)
45	29.41 (+)	6.25 (−)	82.89 (−)	80.06 (−)	55.9 (−)	93.65 (−)	47.86 (+)	32.93 (−)	27.77 (+)	25.81 (−)	40.09 (−)	0.5 (−)	11.41 (−)	37.74 (−)
60	11.11 (−)	4.37 (+)	86.03 (−)	80.66 (−)	60.25 (−)	96.27 (−)	26.49 (+)	46.03 (−)	13.59 (+)	71.91 (+)	58.52 (−)	25.78 (−)	27.69 (−)	39.51 (−)
75	21.39 (−)	93.75 (+)	88.22 (−)	79.28 (−)	83.08 (−)	90.47 (−)	57.9 (+)	48.91 (−)	3.92 (+)	100 (+)	89.89 (−)	31.36 (−)	29.90 (−)	39.71 (−)
90	33.88 (−)	106.25 (+)	89.6 (−)	72.9 (−)	66.98 (−)	98.8 (−)	11.62 (+)	72.13 (−)	9.8 (−)	110.43 (+)	93.01 (−)	42.18 (−)	47.78 (−)	41.72 (−)
105	49 (−)	73.15 (−)	92.37 (−)	73 (−)	71.08 (−)	99.11 (−)	31.92 (−)	51.85 (−)	45.01 (−)	80.4 (−)	95.87 (−)	91.74 (−)	60.3 (−)	90.95 (−)

By mixing the soil with Euphorbia hirta Leaves (5%) and keeping the same percentage of moisture decomposition was determined by the same procedure as above. Decomposition in treated soil at the end of 105 days was 4 1%. The soil left to itself under the same condition lost (in weight) nearly 1 1%. The decomposition of O M in treated soil therefore is 4 1 − 1 1=3 g out of 5 g leaves added. Thus the percentage decomposition of the leaves in the soil under humid condition is 60° The carbohydrates in treated soil and soil alone were estimated by the same method as adopted for leaves. In soil carbohydrates were estimated before and after 105 days only because the soil contains a very small amount of carbohydrates

The organic carbon was estimated by Walkley and Black's rapid titration method (Piper[17]) The total amount of Nitrogen was determined by Salicylic acid method (Loomis and Shull[13]) and the nitrate and ammoniacal nitrogen by the method of Knowles and Watkins[18]

In the following tables Table 1 represents changes of carbohydrates in O M., Table 2 represents changes of carbohydrates in treated soil and Table 3 shows the percentage of organic carbon total nitrogen total carbohydrates mineral nitrogen organic nitrogen at different periods in O M., T S. and Soil. Table 4 represents percentage fixation and decomposition of total carbohydrates, organic carbon, organic nitrogen, nitrate nitrogen Table 5 represents percentage fixation and decomposition in sugars pectic substances, Hemicelluloses non-cellulose polysaccharides and cellulose.

Discussion

Table 5 shows that reducing sugars (obtained from 70-80% ethanolic solution) decomposed to 49% and 73 15% in O M and T S. (Leaves humified with Soil) respectively at the end of 105 days. The decomposition of non-reducing sugars proceeds to the extent of 92 37% and 79% in O M and T.S respectively Tables 1 and 2 show that after 15 days reducing sugars increase in both the cases, this increase in reducing sugars continues upto 45 days in the case of organic matter and upto 105 days in the case of treated soil. This is best explained by considering the role of micro-organisms, which may have attacked the non reducing sugars and splitted them to reducing sugars by some hydrolysing enzyme. It is also concluded that the decomposition of non-reducing sugars proceeds via reducing sugars. In Table 2 after 45 days there is slow and continuous increase in reducing and non-reducing sugars which persists upto 90 days. This may be explained by assuming either the microbial synthesis of sugars by carbon fixing bacteria (which is also supported by the fact that during this period there is an increase in organic carbon content of the T.S. persisting upto 90 days) or th hydrolysis of higher polysaccharides by soil micro-organisms which may have developed during humification Pectic substances obtained by 70-90% ethanol decompose

rapidly in T S than in O M The decomposition is 74 68% and 99·4% in O.M. and T S respectively

Hemicelluloses obtained by 4% NaOH also decomposed more in case of T.S than in O M After 105 days, decomposition in case of O.M and T.S. is 31 92% and 56 85% respectively In Table 1 the hemicelluloses content shows an increase upto 90 days and in Table 2 the increase of hemicelluloses continues upto 15 days. This increase in hemicelluloses is due to the fact that the decomposition of pectic substances to lignin etc. proceeds via hemicelluloses This view is also supported by the work of O Dwyer[17]

The decomposition of carbohydrates extracted by 17 5% NaOH (Non-cellulose Polysaccharides) is 45 04% and 88·4% for the O.M. and T.S respectively at the end of 105 days. The N C.P (Non-Cellulose Polysaccharides) comprised of galactans, arabans, xylans and mannans increase by 165 36% after 15 days as shown in Table 1 which is then followed by continuous decomposition of N C.P In Table 2 N C.P increase by 25 84% after 30 days and then after 60 days by 71·9% and after this period the increase continues upto 90 days. After 90 days of humification there is a sharp decrease in N C.P content of O M as well as T S For explaining the increase from the original N C P content in Tables 1 and 2 it can be suggested that cellulose may have been transformed into N C.P by some unknown microbial activity This view seems to be more reasonable because there is a decrease in cellulose content in the corresponding periods This increase may also be due to conversion of hemicellulosic substance to N C.P

Cellulose extracted by 72% H_2SO_4 decomposed to 95 87% and 94 74% in O M and T.S respectively Here the cellulose decomposition is almost the same in both the cases Tables 1 and 2 conclusively prove that cellulose decomposition which is maximum after 105 days is accompanied by maximum fixation of nitrogen (Table 3) in the same period. This is supported by the view of H L. Jensen and R. J Swaby[19] The maximum decomposition of cellulose is useful for the soil since on decomposition of cellulose colloidal substances are produced which deter the erosion of soil as said by M.S Calaladi[8]

W H Fuller and A F Norman[20] suggested that with the decreasing amount of lignin decomposition of cellulose increases. Our observation in Table 1 supports the same view

The residual fraction of the O M after foregoing solvents 70-80% Ethanol 4% NaOH 17 5% NaOH and 72% H_2SO_4 and hydrolytic agents is taken to be lignin (11 5%) which decomposes by 11 8% at the end of 105 days Decomposition of total carbohydrates excluding lignin in O M and T.S is 66·3% and 80·95% respectively This emphasizes the role of micro-organisms found and produced during humification in the soil. The soil alone does not contain any reducing non reducing and pectic sugars but the total quan-

tity of carbohydrates has been found to be ·039% including hemicelluloses. The decomposition proceeds by 20·5%.

In Table 3 data of organic carbon total nitrogen, mineral and ammoniacal nitrogen as well as C : N ratio are recorded for the same samples. In Table 4 it is observed that in O M. the amount of organic carbon falls by 49·24% in 105 days by self humification of leaves, whereas organic nitrogen increases by 97 68%. The C N ratio is reduced from 15 7 to 4 26 which means the depletion of carbon proceeds quite rapidly or increase in organic nitrogen is in fairly good amounts. The T.S. (Table 4) shows a fall in carbon by 27·04% and organic nitrogen falls by 1 52% and C.N ratio falls from 10 55 to 8·27 which shows that with depletion of organic carbon, organic nitrogen is also converted into mineral nitrogen and that is why there is not much change in C N ratio of T.S. on humification. This slow continuous decomposition of carbon followed by fixation of mineral nitrogen will be helpful for the soil.

The soil when kept at 15% moisture level without adding leaves shows much less variation in the C N ratio (6 55 to 4 75)

The importance of C N ratio may be judged from the observations of Sreenivasan and Subramanyam[21] Acharya[22] Lockette[23] Bal[24] that when C : N ratio of the humifying plant is less than 22, it helps in the mineralisation when added to soil and plant with C N ratio more than that robs the soil of nitrogen. The weed (Euphorbia hirta) under humification has C N 15 7 and mineralization in O.M. during humification is 41 35% and in T.S. it is 158·97%. These observations support the above view of Sreenivasan.

From the above observations it may be concluded that when the leaves decompose either alone or in the soil oxidation of carbon is higher than the decomposition or mineralisation of organic nitrogen. Mineralisation of organic nitrogen is more in T.S which would serve as nutrient favouring the growth of crops. Hence this weed though non-leguminous may be tried as potential green manure for the fertility of the soil.

It may be noted that the amount of organic nitrogen in O M is 2·079% which is higher than 1 8%. This supports the view of Richards and Norman[19] Parberry and Swaby[18] and Acharya[22] in that the mineral nitrogen increases by 159·97% (T.S) when the leaves are humified in the soil for 105 days. According to the observations of Lieschel[11] later on supported by Daji[7] there should be a balanced proportion of the available nitrogen and carbohydrate compounds in the plant material for its rapid decomposition in the soil. The proportion of available nitrogen to total carbohydrates in Euphorbia Hirta leaves, according to our estimations is 2·079 15·488 or 1 7 44 excluding the lignin. The fixation of mineral nitrogen in case of T.S in 158·97%. The results of Jain and Bhattacharya[8] emphasize the importance of Arouta as green manure where the ratio of available nitrogen and carbohydrates is 1 3 13

and the fixation of mineral nitrogen in T.S is 40 51%. Since the fixation of mineral nitrogen and ratio of available nitrogen and carbohydrates is more in our O.M it may b said that more is the ratio of available nitrogen and carbohydrates more would be the fixation of mineral nitrogen thereby Dhudia is a suitable green manure for the fertility of the soil In order to find out the balanced proportion of available nitrogen and carbohydrates work on other parts of Dhudia and some other weeds is in progress

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SOME UNREPORTED HOSTS OF HELMINTHOSPORIUM

S N Chaturvedi and A. K. Koul

Department of Botany B R. College Agra

Helminthosporium, an imperfect fungus of the order Moniliales, is reported to occur on a wide variety of hosts most of which belong to the family Gramineae (Butler 1918 Bulter & Bisby 1931 Butler *et. al* 1960) A good number of cereals, some of the larger millets and fodder grasses are often subjected to leaf spot, the leaf blight or the stripe diseases caused by different species of *Helminthosporium*. In the tribe Maydeae, *Helminthosporium* has so far been reported from *Coix lacryma*, *Zea mays* and *Euchlaena luxurians*[1] only The junior author found this fungus growing on certain hosts in his plant breeding plots. A search through the literature shows that these are new hosts which are therefore, being put on record through the present communication.

Material and Methods

Parts of hosts carrying the fungal infection were stained with cotton blue and mounted in lactophenol. The spores of the fungus on different hosts reported here were measured and microphotographed from temporary preparations.

Observations

The new hosts of *Helminthosporium* are *Euchlaena mexicana* Schrad Var B. R. C. (a luxuriant variety described by Singh & Paliwal, 1960 see also Koul & Paliwal, 1961) *Coix aquatica* Roxb., and a new species of *Coix*[2] Large populations of all these three species growing in the Botanic Gardens of Balwant Rajput College, Agra, displayed symptoms of the leaf spot disease during the months of September to November Most of the plants in each of the three populations were found to have been infected.

Symptoms of the disease in *Coix aquatica* appear mostly in areas restricted to the upper part of the leaf blades in severe cases only the infection is seen to extend to the leaf base. Diseased leaf blades show tiny brown spots surrounded by pale haloes (Fig 1A) which later on elongate further and then turn almost transparent. At a still later stage the affected leaves start drying up along the diseased spots. In the new *Coix* sps. leaves of mature plants develop brown linear spots which are surrounded by a yellow halo (Fig 1B) When the attack is severe, several diseased spots coalesce to form long transparent stripes along which the leaf gets shredded during later stages

The disease in *Euchlaena mexicana* var B R. C. makes its appearance with the development of small brown spots (Fig 1C) which with the passage

1 Syn *Euchlaena mexicana* Schrad.

2. A new species of *Coix* described by Koul & Paliwal (1962) and still under investigation.

of time elongate considerably and fuse to form brown linear streaks that run parallel to the mid rib Severely affected leaves dry and fall off

Though the disease in all the three plants was wide-spread, in no case did it result in the death of the host plant. Low temperature and greater humidity are probably the two most favourable environmental factors for the growth of this fungus.

Spore Characters—Mature spores of *Helminthosporium* are yellow to olive in colour straight or slightly curved and multiseptate. Scrapings from the infected leaves of *Coix aquatica* display spores which are mostly straight deep brown or pale yellow in colour with 2-8 septa The spores taper at both the ends and are broadest at the centre (Fig 2) The size of the spores varies from $100 \times 34\mu$ to $183 \times 58\mu$ and it averages $126 \times 42\mu$.

The spores obtained from the infected leaves of the new *Coix* sps. are deep brown in colour with 2-6 septa (Fig 3) The size of the spores in this case averages $54 \times 16\mu$ ranging between $100 \times 33\mu$ and $300 \times 67\mu$ The spores appear less chisseled and in fact they are broader than those collected from *Coix aquatica* Prominent constrictions appear on both sides of the septa. When infected leaves of *Euchlaena mexicana* var B. R. C. were teased in a drop of lactophenol large masses of spores got released The straight olive coloured spores (Fig 4) with 2-6 septa average $121 \times 43\mu$ in size These spores have more resemblance with those of the pathogen on *Coix aquatica*

The spore measurements of the fungus on the three hosts were compared with other species of *Helminthosporium* (e g *H avenae H carbonum H gramineum, H maydis H nodulosum H oryzae H sacchari, H sativum, H teres H turcicum*, etc. See Gilman (1957) & Sprague (1955)) occurring on various members of the Gramineae. The size of the spores does not seem to resemble any of these species Further studies to find out whether the pathogens under discussion are new species of the fungus are in hand. The present communication is aimed to put on record some hitherto unreported hosts of *Helminthosporium*.

SUMMARY

Helminthosporium has been collected from three forage grasses namely *Coix aquatica*, a new species of *Coix* with 32 chromosomes and *Euchlaena mexicana* var B. R. C. The spores collected from *Coix aquatica* and *Euchlaena mexicana* resemble each other while those collected from the new *Coix* sps are slightly different in size and shape.

ACKNOWLEDGEMENTS

Our most sincere thanks are due to Prof Bahadur Singh Asst Director National Botanic Gardens, Lucknow for going through the manuscript and giving some valuable suggestions. We are also grateful to Dr S P Singh and Dr R. K. Singh Professor of Botany and Principal B. R College Agra respectively for providing us the facilities for work.

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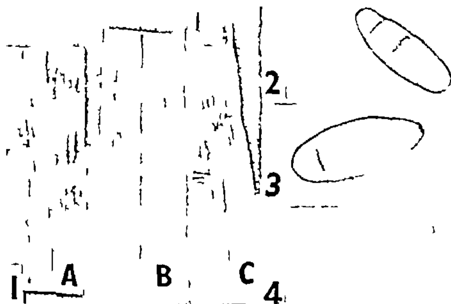


Fig. 1A B & C. Parts of diseased leaf blades of *Coix aquatica*, new *Coix* sps and *Euchlaena mexicana* ar. B. R. C. respectively. Note: yellow diseased spots in A surrounded by a pale yellow halo. B shows prominent brown spots while in C prominent disease spots are noticeable all over the leaf surface.

Figs. 2, 3 & 4. Showing conidia of Helminthosporium on *Coix aquatica*, new *Coix* sps. and *Euchlaena mexicana* ar. B. R. C. respectively X 2160

of time elongate considerably and fuse to form brown linear streaks that run parallel to the mid rib. Severely affected leaves dry and fall off.

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The spores obtained from the infected leaves of the new *Coix* sp. are deep brown in colour with 2-[illegible] septa (Fig. [illegible]). The size of the spores in this case averages [illegible] the range being between 100×[illegible] and 1[illegible]×[illegible]μ. The spores appear [illegible] and in fact [illegible] broader than the [illegible] collected from *Coix aquatica*. [illegible] constrictions appear [illegible] of the septa. When [illegible] leaves of *Euchlaena mexicana* var. B. R. C. were teased in a drop of lactophenol large masses of spores got released. The straight olive coloured spores (Fig. 4) with 2-[illegible] septa average 121×43μ in size. These spores have more resemblance with those of the pathogen on *Coix aquatica*.

The spore measurements of the fungus on the three hosts were compared with other species of *Helminthosporium* (e. g. *H. avenae*, *H. carbonum*, *H. gramineum*, *H. maydis*, *H. nodulosum*, *H. oryzae*, *H. sacchari*, *H. sativum*, *H. teres*, *H. turcicum*, etc. See Gilman (1957) & Sprague (1955)) occurring on various members of the Gramineae. The size of the spores does not seem to resemble any of these species. Further studies to find out whether the pathogens under discussion are new species of the fungus are in hand. The present communication is aimed to put on record some hitherto unreported hosts of *Helminthosporium*.

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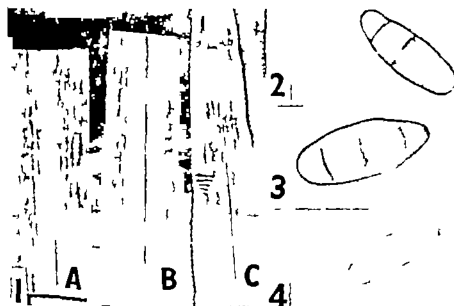


Fig 1A, B & C. Parts of diseased leaf blades of *Coix aquatica* new *Coix* sps and *Euchlaena mexicana* ar B R C. respectively Note: Brown diseased spots in A surrounded by pale llow halo. B shows prominent brown spots whil in C prominent disease spots are noticeable all over the leaf surface

Figs. 2 3 & 4 Showing conidia of Helminthosporium on *Coix aquatica*, new *Coix* sps and *Euchlaena mexicana* var B. R. C. respectively X 2160

A NOTE ON THE DOUBLE PORED DILEPIDID TAPE WORMS OF SOME OF THE INDIAN CARNIVORES

V P GUPTA

Department of Parasitology U P College of Vet Sc & Ani. Husb Mathura

INTRODUCTION

The members of the cyclophyllid family Dilepididae (Railliet et Henry 1909) Lincicome, 1939 with a double set of reproductive organs have been included under its sub-family Dipylidiinae Stiles 1896 which, according to Yamaguti (1959) has the three genera *Dipylidium* Leuckart, 1863 *Diplopylidium* (Beddard, 1913) Lopez Neyra, 1927 and *Joyeuxiella* Fuhrmann 1935

Sondhi (1923) in a study of tape worm parasites of Indian dogs has dealt with the species *D sexcoronatum* Ratz 1900 *D oerleyi* Ratz, 1900 and a third one described by him as a new species *D walkeri*. Another new species from cat, *D catus* was described by Gulati (1929) who gave remarks on the systematic position of *Dipylidium* *Diplopylidium* and *Joyeuxiella* with a list of the species included in these genera According to Southwell (1930) the species parasitic in Indian carnivores were *D caninum* Linnaeus, 1758, *D gervaisi* Setti, 1895, and *D sexcoronatum*. The reports on these tape worms that are subsequently available are of Acharya (1933) about *D caninum* Bhalerao (1935) on *D catus* and *Diplopylidium nolleri* Skrjabin, 1924 Sami (1938) on *D caninum*, Rao (1939) on *Joyeuxia chyzeri* Ratz 1897 from cat, Mudaliar and Alwar (1947) about *Joyeuxiella pasqualei* Diamare, 1893 from cat in Madras and Bhatia *et al.*, (1959) on *D gervaisi* in domestic cat. In these reports, including those of Ramanujachari and Alwar (1954) Thapar (1956) and Rao (1958) the work on tape-worm infestation in dogs and cats is mostly a systematic reporting from morphological considerations and the pathogenic effects evinced in the parasitised animals have received little or no attention

MATERIAL AND METHODS

A collection of tapeworms from dogs, examined both locally and at Gorakhpur two cats and three foxes (*Vulpes bengalensis*) has yielded specimens belonging to the two genera, *Dipylidium* and *Joyeuxiella* The former is represented by the species *D elegantus* Joyeux *et al.*, 1936 and *D caninum* and the latter by *J pasqualei* *D elegantus* does not seem to have been reported from this country and is therefore a first Indian record with a new host record as well. A few salient anatomical features and a brief account of histopathological picture of the intestinal lesion harbouring the embedded scolices in these dilepidid infections and the associated reactions revealed in the surrounding tissue are briefly described in the present note in respect of *D elegantus* and *J pasqualei*

OBSERVATION

The genus *Dipylidium* with a large number of species, parasitises dog cat, wild carnivores and occasionally young children As cited by Wardle and McLeod (1952) the twenty species then known under this genus were revised by Lopez Neyra (1928) who recognised only thirteen species and gave a key for their identification Witenberg (1932) has been stated to believe that there was only one species, *D caninum* which had *D sexcoronatum D oerleyi D walleri, D carracidoi* Lopez Neyra, 1928 and *D porimamillanum* Lopez-Neyra 1928 as its synonyms. This view as stated by Wardle and McLeod, was not accepted by Stewart (1939) while Venard (1938) had recognised under this genus, only three species as valid for which a key was also appended.

Amongst the seventeen species listed by Yamaguti (1959) excepting *D caninum D buencaminoi* Tubangui 1925 *D* *a us D gracilus* Gervais, 1847 *D monticelli* Diamare 1893 and *D otocyonis* and the rest have been named as synonyms of *D caninum* after Witenberg

1 *D otocyonis*

Specimens assignable to this species were collected from one of the three foxes examined post mortem The minute scolex 0 316 in maximum diameter carried a globular rostellum of 0 085 × 0 09 in size and with an armature of rosethorn shaped hooks arranged in four rows—those in first two rows being 25-27 μ long and with the basal disc of 12.9-16 μ in diameter but in the subsequent rows with diminishing size had in the last row the smallest hooks of 11 μ in length with the basal disc of 6. 4 μ diameter The four spherical suckers measured 0 116-0 150 in diameter The neck part is followed by a series of immature segments, broader than long but gradually increasing in length posteriorly The mature segments on the whole, are as long as broad in the beginning but later become longer than broad The gravid segments longer than broad have a length greater than the width The genital pore in mature segment, lies near its middle. The testes, 150-180 in number and lying between the longitudinal excretory canals are 40-90 × 40-70 μ in size. The vas deferens of an extremely coiled character lies anteromedially to the genital pore and the cirrus sac 148-163 × 48-52 μ in size, is situated across the longitudinal excretory canals. The bilobed ovary with many follicles, occupies a total area of 0 10-0 115 × 0 10-0.166 with the vitellaria, lying just posterior to it, occupying 0 077-0 089 × 0.107 -0 115 area In the gravid segment the egg capsules have in each 2-8 eggs and measure 0 048-0 074 × 0 041-0 056 in size and lie scattered between the longitudinal excretory canals The eggs are 14 8-22 μ in diameter

Pathogenicity There is no reference in the available literature about the relation of this tapeworm to the host tissues. The specimens, in very large numbers, were found with their scolices embedded in the mucosa. Histological examination of such patches from serially cut stained sections

revealed that the worms burrowed deep into the mucosa but did not reach the submucous part and the rostellum alone seemed to maintain quite a firm hold on the host tissue. The comparatively weak suckers did not play any prominent part in this direction The harmful effects thus result mainly from the mechanical injury consisting of a marked denudation of intestinal mucosa which, from its ulceration may pave the way for secondary infection. No marked cellular-elements appeared to have infiltrated in and around the areas of attachment

The present form on account of large-sized hooks in the first and second row of rostellum, can not be accommodated in any of the species that have been listed by Lopez Neyra but following Venard, who had characterised *D otocyonis* as having four rows of rostellar hooks which in the first three rows measured 42 26 μ(?? may be 26-42 μ) The present material on this character is identified as belonging to *D otocyonis* which is for the first time being reported from the Indian fox.

The genus *Joyeuxiella* with the genital opening like that in *Dipylidium* is characterised by the testes being less than 90 in number and the egg capsules in the gravid segment containing one egg in each

2. *J pasqualei*.

A large number of specimens belonging to this genus were collected from the two cats available for examination. The worms with scolices deeply embedded in the mucosa had a well-developed rostellum carrying a large number of rose thorn shaped hooks. The histological study revealed the scolices lying deep in the intestinal mucosa, with a fairly stretched out rostellum which appeared to play the main role in effecting a firm hold during attachment, the activity which particularly in heavy infections, may cause to its mucous lining a serious damage consisting of denudation, haemorrhage and destruction of intestinal glands of the area. The comparatively powerful suckers also appeared to assist in effecting the hold as in their cavities plugs of the intestinal mucosa which later became necrosed were observed. No marked cellular reaction was exhibited in the areas of attachment.

The worms, with 250-300 proglottids, measure 250-350 in length and have quadrangular scolex not sharply demarcated from rest of the body and with a maximum width of 0 56-0·71 in the region of suckers which are of 0 15-0 18 diameter The evertible rostellum, with a broader base and narrower anterior region, has a length of 0·11-0 17 and a maximum width of 0 12 0·15 The rostellar armature consists of 14-15 rows of rose thorn shaped hooks which gradually diminish in size posteriorly—the hooks in first row have 11 3-14 5 μ long spines with a basal disc of 9 6-14.5 μ diameter and those of the last row being 6·4-9 4 μ in length and with a basal disc of 6·4-9 4 μ diameter A distinct neck is absent. The proximal segments are broader than long but gradually

increase in length. The mature segments are as broad as long while the more mature and gravid segments are longer than broad. A pair of longitudinal excretory canals run on each side. The genital pore, in the mature segment, lies near the middle but in the immature ones it is slightly anterior in position. The testes, confined between the longitudinal excretory canals, are 90-110 in number and measure 0·07-0·12×0·07-0·08 in size. The extremely coiled vas deferense, before entering the cirrus sac, lies antero-medially to it. The cirrus sac, enclosing a coiled seminal vesicle and a long unarmed cirrus, crosses the longitudinal excretory canal. The bilobed ovary composed of many follicles, occupies an area of 0·3-0·4×0·27-0·33. The compact but lobulated vittellaria lying behind the ovary occupy an area of 0·08-0·12×0·13-0·15. The egg capsules, 48-85 μ in diameter lie scattered in the gravid segment and may even extend beyond the excretory canals. A single egg 41-54 μ in diameter is contained in one egg capsule.

According to Wardle and McLeod (1952) of the ten species included under *Joyeuxiella* by Lopez Neyra Witenberg (1932) had recognised only *J pasqualei* and *J echinorhynchoides* as valid ones remarking that *J gervaisi* and *J dongolensis* Beddard, 1913 were not satisfactorily described and the remaining ones were all identical to *J pasqualei*. Yamaguti (1959) following Witenberg has also recognised only these two species. The specimens recovered from cat have been found to resemble, in nearly all morphological details, the species *J pasqualeiformis* Lopez Neyra, 1928 which was held to be a synonym of *J pasqualei* by Witenberg a view also upheld by Yamaguti. The present finding of *J pasqualei* in cat of this locality extends its distribution to Northern India as well.

(All measurements except otherwise stated are in mm.)

EXPLANATION OF FIGURES

Fig 1 *J pasqualei*—Rostellum
Fig 2 *J pasqualei*—Scolex.
Fig 3 *J pasqualei*—Mature segment.
Fig 4 *J pasqualei*—Gravid segment
Fig 5 *D oligorchis*—Rostellum.
Fig 6. *D oligorchis*—Scolex
Fig 7 *D oligorchis*—Mature segment.
Fig 8. *D oligorchis*—Gravid segment
(All above figures are camera lucida drawings)

Fig 9 Photomicrograph—Cross section of intestine with scolex of *D oligorchis* deeply embedded in the intestinal mucosa showing denudation 190 X.

Fig 10 Photomicrograph—Cross section of intestine with scolex of *J pasqualei* deeply burried and the suckers having mucosal plug in their cavities.

Acknowledgements

I owe a deep sense of gratitude to Professor Dr B P Pande M Sc. D Sc. for his constant supervision, invaluable suggestion and helpful criticisms during the course of this work which forms a part of the thesis approved for the award of M. V Sc. Degree of Agra University Thanks are due to the Principal of the College for the facilities provided and to Dr B C. Mahendra Professor of Zoology Agra College, Agra for supplying the Zoological names of the host animals.

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Fig. 1



Fig. 2

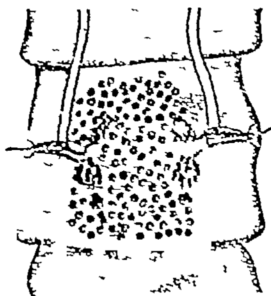


Fig. 3



4



Fig 5

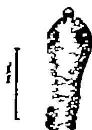


Fig 6

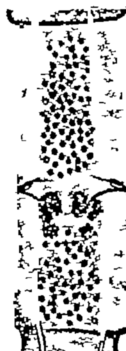


Fig 7



Fig 8



Fig. 9



Fig. 10

Higher concentrations were further responsible for delaying the flower bud formation reducing the percentage fruit-set and causing delayed maturity of fruits, while the control and pinched plants, however produced almost similar performance.

It is apparent from the data, however that the number of fruits per plant was the least under 800 p p m. followed by 400 p. p m. The lowest concentration did not vary with the control and the hand pinched plants. It was interesting to observe that the size of the fruits under the 400 p p. m. was as good as the control, yet the yield per plant was significantly low due to less number of fruits per plant. Size and yield of fruits per plant in the control hand pinched and 200 p p m treatments exhibited negligible variation and were superior to the higher concentrations Little variation was obtained in the percentage of total solids and moisture per fruit under each treatment.

The above findings suggest that higher concentrations of MH are generally injurious to growth and yield of brinjal. It is still possible that lower concentrations of MH may be helpful in increasing the average number of lateral shoots and the yield per plant.

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TABLE 1

The effect of different concentrations of MH and manual pinching on growth and yield of Brinjal (110 days after treatments)

MH concentrations in parts per million.	Height of main stem in cm	Diameter of main stem in cm	Average number of branches per plant	Average length per branch in cm	Average diameter per branch in cm	Average spread per plant along the rows	Spread per plant across the row	Number of days required for flower bud formation	Percentage fruit set.	Average time required for maturity of fruit	Average length per fruit in cm	Average diameter per fruit	Number of fruits per plant	Average yield per plant in Kgm	Percentage of total solids	Moisture percentage
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Control	66 67	2 38	10 50	58 50	1 34	122 60	133 65	43	54 47	51	20 11	4 39	16 25	0 9425	10 77	89 83
Pinched	29 34	2 42	12 80	60 50	1 60	125 37	138 50	41	54 86	52	22 28	5 02	17 45	0 9175	10 73	89 27
200	74 43	2 33	12 60	54 90	1 39	115 78	135 50	45	47 38	59	21 55	5 05	17 35	0 9525	10 70	89 21
400	66 17	2 23	10 80	57 60	1 38	113 52	104 17	53	44 42	61	20 77	5 02	13 33	0 8200	10 70	89 30
800	53 95	2 20	9 80	41 80	1 29	104 10	99 70	59	34 87	71	16 83	3 85	8 50	0 4650	10 63	89 35
C.D. @ 5%	20 521	1 33	0 646	22 48	0 147				—		1 66	1 96	3 25	0 1761		

Effect of MH on the growth of Brinjal plants



Fig 1 — Control



Fig. 2 — 200 p p. m.



Fig. 3 — 800 p. p. m. showing the stunted growth of the main shoot

EFFECT OF SIMPLE SURFACTANTS ON KINETICS OF ELECTRODE PROCESSES

H P AGARWAL

Professor of Chemistry Motilal Vigyan Mahavidyalaya Bhopal

INTRODUCTION

A sinusoidal current causes the potential of an electrode at which a fast charge transfer process occurs, to vary periodically about the equilibrium potential. The resulting alternating current includes a rectification component which is caused by the asymmetry of polarization curves This kind of rectification was at first reported by the name— rédoxokinetic effect [1] but now it is popularly known as faradaic rectification[2,3] The theoretical treatment[4,5] of the method enables the determination of transfer coefficient and standard rate constant of the electrode reaction Making use of an amplitude-modulated radio-frequency polarizing current Barker[6] has recently developed a radio-frequency polarography i e RF technique which enables the quantitative estimation of as low as 10^{-10} gm of metallic ion in 01 c. c of the solution. The faradaic rectification method is very helpful in studying the mechanism and kinetic of electrode processes. It has been used in the present work for studying the effect of simple surfactants on transfer coefficient and kinetics of ferrous-ferric redox processes, as they (simple surfac tants) have been reported[7] to influence the chang in redoxokinetic potential

EXPERIMENTAL

The alternating current was made incident on two polished bright platinum foil electrodes of the cell and the rectification component developed at one of them (earthed electrode) was measured with respect to a third polished bright platinum foil electrode dipped in the same solution For obtaining reproducible surfaces[8] all the three electrodes were first washed with chromic acid and then with distilled water The electrodes were then dipped in Absolute alcohol and were heated red hot in (spirit lamp) flames The process was repeated twice

The other particulars about the type of cell used the disposition of the three electrodes with respect to each other the circuit diagram and the method used for measurement of ϕ, the redoxokinetic potential and V the alternating voltage incident are the same as described earlier[9] The simple surfactants chosen for the study were isopropyl alcohol, acetone, isobutyl alcohol, amyl alcohol and chloroform—all of them were of Analar grade. For studying the effect of simple molecules on transfer coefficient of the ferrous-

Runnicles[10] It is found to be $9{\cdot}2 \times 10^{-6}$ cm^2 $sec.^{-1}$ Same order of value of the diffusion coefficient was obtained when the diffusion of 001 M ferrous ammonium sulphate under similar conditions was studied instead. The change in redoxokinetic potential ψ is not being influenced with the variation in temperature[9] or when the concentration[11] of each of the reactants is being changed from 0 002 M to 0·001 M Therefore the value of transfer coefficient at 35°C for ferrous ferric redox couple (concentration of each cation being ·001 M containing 0·4 gm mol/litre of acetone) can be calculated from the values of ψ given in Table I for the reactants (concentration of each being ·002 M) containing 0·4 gm. mol/litre of acetone at 19°C On substituting the value of α and D at 35°C, and ψ at 200 cycles/sec at 4 mv of a. c., the standard rate constant K_s is determined from the theoretical equation[8] applicable at low frequencies of a c. i. e.

$$K = (5 - \alpha)\frac{V^2 nF}{4\psi RT}\sqrt{\frac{w\, D}{2}} \qquad (?)$$

The value of K_s thus obtained is ·055 cm. Sec^{-1} On comparing it with the value of K obtained[11] when no acetone is being added to the redox system, it is found to be approximately 20% higher It is curious to note that the rate constant increases on addition of surfactants, inspite of the fact that α had decreased.

SUMMARY

The addition of simple surfactants to ferrous ferric redox couple either increases or decreases the transfer coefficient which is being determined by the faradaic rectification method. The extent of increase or decrease of transfer coefficient depends upon the nature of the surfactant added rather than their concentrations. When chloroform acetone iso-butyl alcohol and iso-propyl alcohol are added to the ferrous-ferric redox couple (each having 0·002 M concentration in 1 N H_2SO_4) the transfer coefficient of the redox process decreases and the values of transfer coefficient obtained in presence of each of the surfactants are 575 569 556 and 541 respectively at 19°C Therefore on addition of the above substance the rate of cathodic reaction decreases in the order —

Iso-propyl alcohol < iso-butyl alcohol < acetone < chloroform. When amyl alcohol is being added to the redox system the transfer coefficient increases and its value is 613 at 19°C. Hence in presence of amyl alcohol, the reaction during the cathodic half wave is enhanced. When 0 4 gm. mol/litre of acetone is added to the ferrous-ferric redox system (cocentration of each cation being 001 M in 1 N sulphuric acid) th rate constant K_s is 055 cm sec^{-1} at 35°C. The rate constant so determined is about 20% higher as compared to that obtained without addition of acetone.

TABLE 1

Dimensions of bright polished platinum foil electrodes used.

a. c. electrodes	length in cms.	breadth in cms.
1	1 30	1 15
2.	1 30	1 10 (earthed)
Reference electrode	1 30	1 10

Temperature of the thermostat = 19° ± 05 C

S. N	Frequency of a. c. used in cycles/sec	Values of ψ redoxokinetic potential in micro-volts at 4 millivolts of a. c. when concentration of oxidant and reductant each is ·002 M in 1–N H_2SO_4 containing				
		0·52 gm. mol per litre of acetone	0·52 gm. mol per litre of isopropyl alcohol	Isobutyl* alcohol	chloro-form	amyl alcohol*
1	50	–8	–6	–6	–6	–14
2	100	–11	–8	–9	–8	–20
3	200	–15	–11	–13	–12	–28
4	500	–20	–13	–17	–18	–36
5	1000	–22	–13	–18	–24	–36
6	2000	–22	–13	–18	–24	–36

* gm. molar concentration of the species is 1/119 5th of that of its solubility

TABLE 2

Values of concentration gradient and of mean concentration for diffusion of 001 M ferric ammonium sulphate in 1—N sulphuric acid solution containing 0·4 gm. mol/litre of acetone.

Volume of ferric ammonium sulphate solution (prepared in 1—N sulphuric acid containing 0·4 gm mol of acetone) taken in compartment A of the porous diaphragm cell = 60 c. c.

Volume of supporting electrolyte (0·4 gm. mol/litre of acetone in 1—N H_2SO_4) taken in compartment B of the porous diaphragm cell = 110 c.c.

TABLE 2 (*Contd*)

Characteristic constant of the cell = 3·46

Temperature of the thermostat = 35° ± ·05 C

S N	Time t in minutes	Concentration of the diffused salt in gm Equ. per litre $C_1 \times 10^4$	Time t_2 in minutes	Concentration of the diffused salt in gm. eq per litre $C_2 \times 10^4$	Concentration gradient $\frac{dc}{dt} \times 10^4$	Mean concentration $\bar{C} \times 10^4$
1	237	4 4	600	13.8	·026	9 1
2	260	5 2	570	13.5	026	9·3
3	282	5 8	535	13 3	03	9 5
4	308	6 0	506	12 0	·03	9·0
5	330	6 3	474	11.5	·036	8·9
6	361	7 1	444	10.2	·037	8·6
				Mean	031	9·0

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SHARPNESS OF THE COLLOID-ELECTROLYTE BOUNDARY AS A CRITERION TO MEASURE THE MOBILITY OF COLLOID MICELLES II ELECTROPHORESIS OF $Cr(OH)_3$ SOL

P D BHATNAGAR AND ABANI K. BHATTACHARYA
Department of Chemistry Agra College Agra

Studies on the migration of colloid electrolyte boundary of $Fe(OH)_3$ sol with various equiconducting supernatant electrolytes were communicated previously in the light of the Kohlrausch Weber theory for ionic boundaries. It has been discussed in the foregoing paper (1962) that the choice of a suitable supernatant liquid to produce a sharp descending boundary of the positively charged $Fe(OH)_3$ sol would give a more probable value of the electrophoretic velocity of colloidal micelles when they behave as leading ions.

This paper deals with our observations on the colloid-electrolyte boundary of $Cr(OH)_3$ sol and various equiconducting electrolytes used as supernatant liquids. The colloid electrolyte interaction, as reported in our previous paper dealing with $Fe(OH)_3$ sol-electrolyte boundary appears to be more pronounced in this case. The electrophoresis of $Cr(OH)_3$ sol further confirms our earlier views that the electrophoretic velocity in the descending limb would give a more representative value, and the anology between purely ionic and colloid-electrolyte boundaries is subject to certain conditional factors.

Experimental

The apparatus consits of two parts after Tiselius pattern, the electrode vessel and the main U tube fitted by standard glass joints at P_1 and P_2. M_1 and M_2 are the electrodes supplying a constant current, the device for maintaining a constant current is described below —

A sharp-cutoff pentode 6 S J 7 whose plate current is independent of its plate voltage within a range of 40 to 500 volts (R.C.A. Receiving tube manual p. 0.211) is used in series with the U tube as shown in the circuit.

The voltage drop across the whole U tube is first of all determined by the potentio-V T V M system allowing the required current (0 1 to 0.4 milliamps) to flow through the circuit. The supply voltage from the rectifier is so adjusted that the plate voltage remains sufficiently above 40 volts. Now because of the characteristics of the tube 6 S J 7 any change in the voltage drop across the U-tube due to changes of the resistance will not change the current in the circuit as long as the plate voltage remains between 50 to 500 volts The grid of the valve is connected to a series of batteries

to give it the required negative potential for the desired amount of current (0 1 to 0 4 milliamps)

Observations & Discussion

With equiconducting solutions of NaCl KCl $BaCl_2$ and $AlCl_3$ as supernatant liquids it will be seen in the Plate no 2. that the descending as well as the ascending boundaries are quite diffuse but with the equiconducting solution of LiCl the descending boundary is comparatively sharp, while the ascending one is quite diffuse. Gradually a sharper boundary of deeper intensity is formed inside the diffuse ascending boundary and the intense layer tends to overtake the diffuse one. The ionic velocities of Li^+ Na^+ K^+ Ba^{++} and Al^{+++} and the characteristic sharp or diffuse boundaries have been given in the Table I The mobility of $Cr(OH)_3$ sol micelles as calculated from the sharp descending boundary with LiCl as supernatant liquid is given in Table no. 2.

The formation of a deep boundary inside the diffuse ascending boundary (Plate no 1 $Cr(OH)_3$-equiconducting LiCl supernatant liquid) suggests the effects of colloid-electrolyte interaction which may be visualised as follows —



$$Cr(OH)_3 + LiCl \quad Cr(OH)_2Cl + LiOH$$

In consequence of such possibilities the sharpness appearing in the ascending limb may be produced by the micelles of $Cr(OH)_2Cl$ which may be faster than the $Cr(OH)_3$ sol particles

In the light of the Kohlrausch-Weber theory the sharpness is produced in the ascending limb when the colloidal particles move with the same velocity as the leading ion. In the descending limb colloid micelle acts as a leading ion and the sharpness would appear when the ion of the supernatant liquid would move with the speed of the colloid micelle. The velocity of the $Cr(OH)_3$ micelles calculated from the electrophoretic data lend support to our view that the more probable velocity of the colloidal particles would be given by the electrophoretic velocity of the descending boundary The value of the velocity of the sharp boundary in the ascending limb is the representative value of the ionic mobility constituting the supernatant layer

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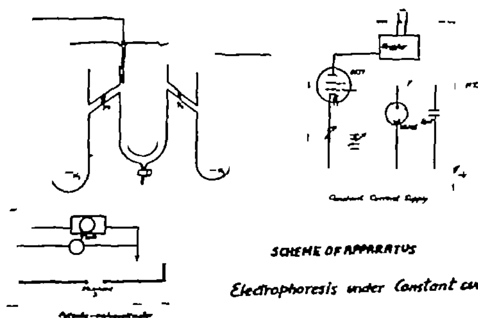
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TABLE 1

Equiconducting supernatant liquid	Ascending boundary					Descending boundary				
	LiCl	NaCl	KCl	$BaCl_2$	$AlCl_3$	LiCl	NaCl	KCl	$BaCl_2$	$AlCl_3$
Mobility of the cation (Temperature 25°C)	39	56	73	63		39	56	73	63	--
Cr(OH) sol boundary	diffuse (deep boundary forms inside the colloid layer to overtake the diffuse one)	diffuse	diffuse	diffuse	diffuse	comparatively sharp	diffuse	diffuse	diffuse	diffuse
Plate No	1	2	2	2	2	1	2	2	2	2

TABLE 2

Particl	Li^+ in LiCl	$Cr(OH)_3$	Na^+ in NaCl	K^+ in KCl	Ba^{++} in $BaCl_2$	Al^{+++} in $AlCl_3$
Mobility 10^5 (cms/sec/unit pot. grad.)	39	57	58	76	63	—



SCHEME OF APPARATUS

Electrophoresis under Constant current

mts 0 20 40 60 **PLATE NO 1** 0 20 40 60

Ascending boundary — Descending boundary

$Cr(OH)$ Sol – Equiconducting LiCl supernatant liquid

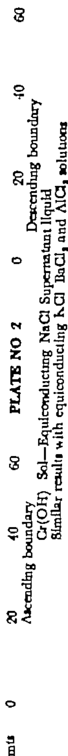


PLATE NO 2

$Cr(OH)_3$ Sol—Equiconducting NaCl Supernatant liquid
Similar results with equiconducting KCl $BaCl_2$ and $AlCl_3$ solutions

STUDIES ON THE ELECTROPHORETIC VELOCITY OF COLLOIDAL PARTICLES BY BOUNDARY METHOD

[PART V—SIGNIFICANCE OF THE MAXIMA IN THE ELECTROPHORETIC VELOCITY CONDUCTIVITY CURVE OF SOLS DURING DIALYSIS IN THE LIGHT OF THE EQUATION $c=a+\frac{m \quad 1/t}{n+1/t}$ FOR SLOW COAGULATION]

ARVIND KUMAR AND ABANI K. BHATTACHARYA
Agra College Agra

SUMMARY

The cataphoretic velocities of $Fe(OH)_3$ sol have been studied at different stage of dialysis taking various equi-conducting chlorides as the super natant liquid On plausible grounds it has been discussed that the peak of the U-conductivity curves (or ζ-c curves) corresponds to the critical stability concentration a of the equation $c=a+\frac{m \quad 1/t}{n+1/t}$

INTRODUCTION

It had been observed by Kruyt[1] Freundlich and Rona[2] that the effect of electrolyte having Univalent inorganic cation was to increase the ζ-potential at first and then to decrease with increasing electrolyte concentration after the maxima was reached The experiments of von Elissafoff and of Freundlich and Rona[3] further showed that the cations of the heavy metals such as silver and lead had a stronger lowering effect than the light metals and that the basic dyes such as new fuchine and crystal violet are particularly active due to which the charge of the capilaries was reversed at a quite small concentration. The valency of the cations was also observed to have very remarkable effect on the ζ-potential with the increase or decrease in the concentration of the electrolyte. The effect of increasing and decreasing the concentration of electrolytes on the zeta potential of the sol particles may be conveniently shown by measuring the cataphoretic velocity U of dialysed sols at regular intervals. Since in the sample of the sol to be dialysed the electrolyte concentration is ordinarily higher than the peak point in the U-c curves, the electrophoretic velocity and hence the zeta potential would increase upto a maxima and then would decrease as the concentration of the elec trolyte falls below the concentration at the peak point.

This has been actually observed in our studies on the effect of dialysis on the electrophoresis of Ferric hydroxide and chromic hydroxide sols under constant current with equiconducting chlorides used as the super natant liquid over the boundary in a U-tube. It has been observed by ploting the electrophoretic velocity against the specific conductivity of the

dialysed samples that the zeta potential increases upto a certain stage of dialysis to a maxima after which there is a fall in the electrophoretic velocity if the dialysis is continued further i e., beyond the peak point. On certain assumptions it has been possible to show (see later) that such observations are due to the inverse relation of the electrophoretic velocity U of the sol particles with the ionic strength $\frac{1}{2}\Sigma CZ^2$ of the solution upto the dialysed stage of the sol corresponding to the maxima, when it may be assumed that the ionic environment of the particle as counter ions has reached the stage of limiting equilibrium with the charge on th colloidal particles

Kumar and Bhattacharya and Bhattacharya[4] in their study of slow coagulation of sols evolved an equation correlating the electrolyte concentration and the time of coagulation which was expressed in the form $C=a+\frac{m\,1/t}{n+1/t}$ where a, m and n are parametric constants. They interpreted these constant and explained that the constant a was a very important quality which was defined as the concentration of the precipitating electrolyte which the sol can stand without being coagulated for an infinite time Thus the effective concentration of the electrolyte to congulate the sol within a measurable time was c a where C was the concentration of the electrolyte added Hence the value of the quantity 'a which is specific for the sol and the electrolyte may be considered to be of greatest significance in determining the limit of the stability of the sol in relation to the thickness of the double layer and hence of the ξ-potential ranging from the critical state of the neutral particles to that of the stable colloidal micelle. This stable state may be governed by the ionic strength as well as the adsorption equilibrium maintained within the limit of the critical stability concentration a of the precipitating ion Thus it may be visualised that during dialysis the ionic strength being higher than the critical stability concentration a the electrophoretic velocity (or ξ-potential) will gradually increase upto a maxima and then the curve will show a fall as the ionic strength of the solution decreases below that represented by the peak of the curve. This peak therefore suggested a very significant characteristic of the state of the sol with different amounts of electrolyte At the peak of the curve $d\xi/dc$ must be zero which can be interpreted as the state of equilibrium between the change of the sol particles and the concentration of the counter ions of the ionic environment which should be proportional to the ionic strength of the solution It may thus be plausibly assumed that the concentration of the electrolyte at the peak represents the critical stability concentration a of the electrolyte for the sol.

The present paper deals with the studies of electrophoresis of $Fe(OH)_3$ sol at its different stages of dialysis taking various equiconducting chlorides having uni and bivalent cation as the supernatant liquid.

Experimental

$Fe(OH)_3$ sol was prepared by adding concentrated ammonium carbonate solution to a solution $FeCl_3$ with constant stirring until the precipitate just

ceased do dissolve The precipited ferric hydroxide was then dissolved by adding just the sufficient quantity of 5% $FeCl_3$. The sol was then dialysed in a parchment paper bag against running distilled water

The apparatus used for determining the electrophoretic velocity of the sol, consists of two parts after Tiselius Pattern The electrode vessel and the main U tube were fitted by standard glass joints at P_1 and P_2 (vide figure 1) M_1 and M_2 are the electrodes to maintain a constant current in the circuit with the help of a sharp cut off pentode 6 S J 7 The sharp cut off pentode 6 S J 7 whose plate current is independent of its plate voltage within a range of 40 to 500 volts (R. C A Receiving tube manual p 0211) is used in series with a U tube as shown in the circuit.

The voltage drop across the whole U tube is first of all determined by the potentio V T V M system allowing a constant current (0·1 to 0 4 milliamp) to flow through the circuit. The supply voltage remains sufficiently above 400 volts Now because of the characteristic of the tube 6 S J 7 and change in the voltage drop across the U tube due to change of the resistance will not change the current in the circuit as long as the plate voltage remains between 40 to 500 volts. The grid of the valve is connected to a series of batteries to give it the required negative potential for the maintenance of the definite amount of current

Observations

Table 1

Cataphoretic velocity and Sp conductivity at different Stages of dialysis of Fe(OH) sol

Supernatant liquid Equiconducting LiCl solution.

Duration of dialysis	Sp conductivity in mhos	Vel of Ascending boundary U in cm / Sec./Unit Pot. Grad.	Vel. of Des. boundary U in cm /Sec. /Unit Pot. Grad
7 days	$1\ 8\times10^{-4}$	23×10^{5}	31×10^{5}
10 ,,	$1\ 18\times10^{-4}$	30	36
12 ,,	$0\ 82\times10^{-4}$	46	51
13 ,,	$0\ 69\times10^{-4}$	39	40
15	0.59×10^{-4}	32	33

TABLE 2

Cataphoretic velocity and Sp cond at different stages of dialysis of $Fe(OH)_3$ sol
Supernatant liq Equiconducting NaCl

Duration of dialysis	Sp Conductivity in mhos	Vel. of Ascending boundary U, in cm/Sec/Unit Pot Grad.	Vel. of Des. boundary U in cm/Sec/Unit Pot. Grad.
7 days	1.8×10^{-4}	33×10^{4}	36×10^{4}
10	1.18×10^{-4}	41	43
12 "	0.82×10^{-4}	53	58
13	0.69×10^{-4}	40	50
15	0.59×10^{-4}	36	38

TABLE 3

Cataphoretic velocity and Sp conductivity at different Stages of dialysis
Supernatant liquid Eq conducting $BaCl_2$ solution

Duration of dialysis	Sp Conductivity in mhos	Vel of Ascen boundary U in cm/Sec/Unit Pot Grad.	Vel of Des. Boundary U in cm/Sec/Unit Pot. Grad.
7 days	1.8×10^{-4}	35×10^{4}	19×10^{4}
10	1.18×10^{-4}	46	23
12	0.82×10^{-4}	65	30
13	0.69×10^{-4}	58	"6
15	0.59×10^{-4}	50	22

DISCUSSION

It will be seen (Table 1 curve 4) that the values of U or ζ-potential gradually increases upto a maxima at different stages of dialysis from A to B and the potential falls from B to C as evidenced by the electrophoretic velocity-conductivity curve They are similar to the ζ-C curves of Freundlich, Ross

and others *loc. cit.* These observations are in agreement with those of the previous authors under reference, although the modus operandi is differrent in our case. The peak of the curve has been found to appear in the case of many sols and hence it suggests a very significant characteristic of the state of the sol where $d\xi/d\kappa$ become equal to zero. This can therefore be interpreted as the state of equilibrium between the charge of the sol particles and the concentration of the counter ions in the ionic environment which mainly depends upon the ionic strength of the system. This equilibrium at the peak point may therefore be plausibly assumed to be connected with the critical stability concentration of the electrolyte for the sol under investigation. The inverse relation between the ionic strength and the electrophoretic velocity or ζ-potential may be deduced on the assumption that the environment of the counter ions around a charged colloid particle is either identical with or very closely similar to the ionic atmosphere of opposite charge around a single ion.

When the electrophoretic velocity becomes constant it may be assumed that the potential at the boundary has been stabilised. The electrophoretic velocity U according to Helmholtz and Smoluchowski[6] is given by —

$$U = \frac{\theta \zeta E}{4\pi\eta} \qquad (1)$$

where θ is the dielectric constant.
ζ the zeta potential,
E—the field strength and
η—the viscosity of the medium.

It is also known that the charge density

$$\sigma = \frac{\theta \zeta K}{4\pi} \qquad (2)$$

Where

$$K = \frac{1}{d} = \sqrt{\frac{8\pi\epsilon^2 N^2 \Sigma c z^2}{1000\ DRT}} \qquad (3)$$

according to Debye and Huckel and d thickness of the double layer ϵ the electronic charge, N the Avagadro number and D the electric constant and $\Sigma c\, z^2$ is the ionic strength connecting equations (1) (2) and (3) the relation between the electrophoretic velocity U and ionic strength is given by equation :—

$$U = \frac{E\sigma}{\eta} \sqrt{\frac{1000\ DRT}{8\pi\epsilon^2 N^2 \Sigma c z^2}} \qquad (4)$$

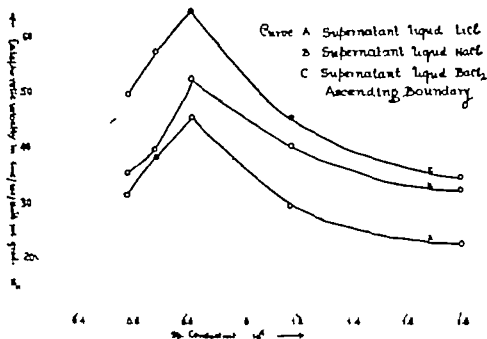
Now since the value of U becomes in steady in electrophoresis after passing the current for a certain interval it may be assumed to indicate that the charge density σ remains constant. If the field strength E, (under constant current) the viscosity and the dielectric of the medium are also assumed to remain constant it directly follows from the above derivation that U is inversely proportional to the variable $\Sigma c z^2$. Under such conditions the portion AB

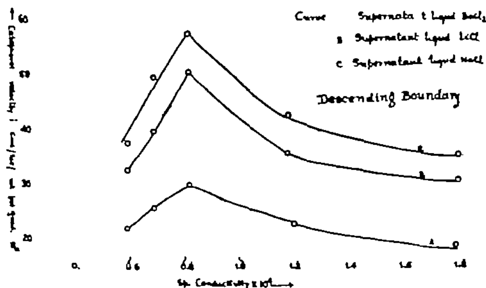
of the curve can be explained till the limiting equilibrium between the charge on the colloid particle and its counter ions in ionic environment of the double layer is reached at the peak of the curve. Further lowering of concentration of the electrolyte by dialysis may disturb this equilibrium by the changes in the concentration of the ions in the double layer which results in the lowering of the charge density After this stage U depends upon the ratio of $\sigma/\Sigma CZ^2$ The portion BC of the curve represents that the lowering of σ has a greater effect on U than that of ΣCZ^2 Hence U gradually falls after the peak point. If these assumptions are admissible the interpretation of the peak point becomes possible and the connection between the constant a in the foregoing equation and the concentration of the electrolyte at the peak point can be realised

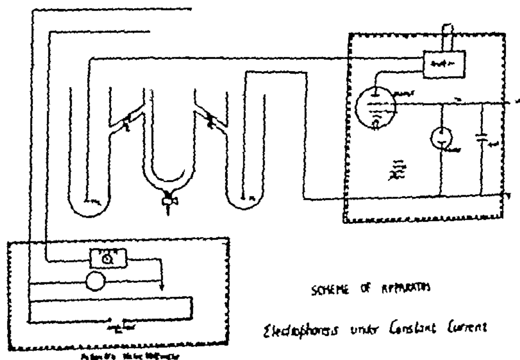
Further work is in progress

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Curve A Supernatant liquid LiCl
B Supernatant liquid NaCl
C Supernatant liquid BaCl2
Ascending Boundary
60
50
40
30
20


Curve Supernatant liquid BaCl2
B Supernatant liquid LiCl
C Supernatant liquid NaCl
Descending Boundary
60
50
40
30
20



SCHEME OF APPARATUS

Electrophoresis under Constant Current

STUDIES ON THE COAGULATION OF ANTIMONY AND GOLD SOLS BY LIGHT EXTINCTION TECHNIQUE AT THE SAME STAGE OF COAGULATION GIVEN BY THE SAME EXTINCTION VALUE

ARVIND KUMAR AMAL KUMAR & ABANI K. BHATTACHARYA
Agra College Agra.

SUMMARY

Antimony and gold sols were prepared and the relation between electrolyte concentration and time of coagulation was studied with KCl $BaCl_2$ and $AlCl_3$ in the light of Bhattacharya's equation by employing the light extinction technique. Different concentration of the electrolyte were added the times for a particular stage of coagulation given by the same extinction value were graphically determined. The plot of l/c-a and t for the same stage of coagulation was found to be linear in agreement with the equation

$$c = a + \frac{m.l/t}{n + l/t}$$

INTRODUCTION

Extensive studies of coagulation have been carried out earlier by many workers such as Smoluchowski[13] Freundlich[6] Zsigmondy[16] Weiser[14] Westgren[15] Kruyt and Van Arkel[9] Dhar and Ghosh[3] Mukherjee[11] and Joshi[8] Inspite of their extensive work the fact remains that no suitable expression is found in the literature which correlates the concentration of the electrolyte with the time of slow coagulation.

Mathew and Murphy[10] Dumanski and Scherschnev[4] while investigating the relation between the electrolyte concentration and the stability of sols brought forward the important fact that time of coagulation is a function of the electrolyte concentration which as expressed by Dumanski was of the form t=ac where t is the time of coagulation and c is the no of c.c.s of the electrolyte added, a and n being constants for a given system. Mathew and Murphy further found that for every electrolyte there was a limiting concentration below which coagulation was not possible.

Bhattacharya and coworkers[1] made an interesting study of the relation between the concentration of the electrolyte and the time of coagulation. They suggested a relation between c and t which is expressed as $c = a + \frac{m.l/t}{n + l/t}$ where a, m and n are constants. The constant a is determined graphically by ploting c against l/t, and then the plot of l/c-a and t is found to be linear . F..

Recently Ghosh B N[9] discussed the kinetics of coagulation in the light of Smoluchowski's equation and also the theory of stability of lyophobic colloids in terms of the energy of repulsion V_R and the stability ratio W which increases with the particle radius r. He further assumed with plausible reasons that the θ factor in the Smoluchowski's equation for slow coagulation was proportional to I/W or I/r. He deduced by approximation that θ of Smoluchowski's equation and the time of coagulation can be represented by the equation —

$$\theta = \frac{K}{1+at}$$

where K includes n_0 (initial no. of particles per c.c.) and the proportionally constant, and a represents α/T in Smoluchowski's equation where α is another constant, assumed by Ghosh in the equation $\theta \propto \Sigma n \propto \frac{n_0}{1+\alpha t/T}$

In a later communication Ghosh[7] deduced Bhattacharya's equation, $1/c\text{-}a = \frac{n}{m} t + \frac{1}{m}$ from the Reerink's equation for an colloid and electrolyte which was expressed as $\log W = -(\alpha + \frac{1}{2}) \log c - \log K$ where K stood for all the constant terms in the expression worked out by Verwey and Overbeek[13] the plot of log W against log c was shown by Reerink to be linear. Similarly the plot of 1/c a and t is found to be linear according to Bhattacharya's equation. Since Reerink's equation has got a theoretical basis hence the validity of Bhattacharya's equation on theoretical grounds could be justified.

In this paper we communicate our observations on the slow coagulation of antimony sulphide and gold sols by the light extinction technique in the light of the equation $c = a + \frac{m.1/t}{n+1/t}$

Experimental and Observations

The antimony sulphide sol was prepared by passing slow bubbles H_2S into a little distilled water in which a solution of potassium antimony tartrate was dropped from a burette. Excess of H_2S was removed by passing a current of purified hydrogen. The sol was then carefully dialysed in a parchment paper bag.

Gold sol was prepared by heating 120 to 150 c.c. of distilled water in a 300 c.c. beaker. 1 c.c. of gold chloride solution (1%) was added and then 2·5 to 3 c.c. of M/5 solution of extra pure potassium carbonate. As soon as the solution came near boiling it was stirred and 2 to 3 c.c. of dilute formaldehyde solution (1 c.c. of commercial 40% formalin to 100 c.c. of water) was added quickly and the mixture was removed from the burner. Reduction was complete in about a minute. The resulting sol was ruby red and transparent to light. It was then dialysed in a parchment paper bag.

TABLE 1

Coagulation of Sb_2S_3 sol with KCl
Concentration of the sol—0·092 gms of Sb_2S_3 per litre

Conc. of KCl in m moles →	62·5		60·0		57·5		55·0		52·5		50·0	
	Time	Ex.	Time	Ex	Time	Ex	Time	Ex	Time	Ex	Time	Ex.
	1·5	53	1·50	51	1·0	48	1·0	46	1·5	45	3·0	45
	3·0	54	2·0	52	1·5	49	2·0	47	2·5	46	5·5	46
	4·5	55	3·5	53	2·0	50	3·5	48	4·0	47	9·0	48
	7·0	56	7·0	54	3·5	51	7·5	50	5·5	48	14·0	49
	10·0	57	10·0	55	6·5	52	11·5	51	9·0	49	21·0	50
	12·5	58	14·0	56	9·0	53	17·0	52	14·0	50	..	...
	16·0	59	20·0	57	15·0	54	22·5	53	21·5	51		
					23·5	55	33·0	54				
t for 48 Ex from graph	...		0·30		1·25		3·00		5·50		9·00	
1/t			2·00		8·00		0·33		0·18		0·11	
a in m moles	20·0		20·0		20·0		20·0		20·0		20·0	
1/c-a	0·023		0·025		0·026		0·028		0·030		0·033	

m=40·0 m moles —80·0

TABLE 2

Coagulation of Sb_2S_3 sol with $BaCl_2$
Concentration of the sol—0.092 gms of Sb_2S_3 per litre

Conc. of $BaCl_2$ in m. moles →	1.8		1.7		1.6		1.5		1.4		1.3	
	Time	Ex.	Time	Ex.	Time	Ex.	Time	Ex.	Time	Ex.	Time	Ex.
	0.5	48	0.5	46	1.0	45	1.25	44	2.5	44	3.5	44
	0.6	49	0.75	47	1.5	46	2.25	45	3.5	45	5.25	45
	1.0	50	1.25	48	2.0	47	3.00	46	4.5	46	7.00	46
	3.0	51	1.75	49	2.73	48	4.00	47	3.75	47	9.50	47
	6.0	52	3.00	50	3.50	49	5.00	48	8.00	48	13.00	48
			6.00	51	5.50	50	8.00	49	1.00	49	19.00	49
			9.00	52	8.5	51	12.00	50	24.00	51	25.50	50
					12.0	52	19.00	51				
t for 48 Ex. from graph.	0.5		1.25		2.75		5.0		8.0		13.0	
1/t	2.0		0.8		0·363		0.5		0.125		0.076	
n in m. moles	1.0		1.0		1.0		1.0		1.0		1.0	
1/c−n	1.250		1.428		1.666		2.000		2.50		3.333	

m = 0·84 m. moles n = 1.36

TABLE 3

Coagulation of Sb_2S_3 sol with $AlCl_3$
Concentration of the sol—0 92 gms. of Sb_2S_3 per litre

Conc of $AlCl_3$ ⟶ in m moles	0550		0500		0460		0443		0407	
	Time	Ex	Time	Ex	Time	Ex	Time	Ex	Time	Ex
	0 50	48 *	1 0	44	1 00	44	1 5	45	2 00	44
	0 15	49	1 25	46	1 50	46	2 25	46	2 25	45
	1 25	50	2 00	49	2 25	47	3 00	47	3 75	46
	3 50	51	3 00	50	3 00	48	4 50	48	5 00	47
	9 00	53	9 00	51	6 00	49	8 50	49	7 25	48
			13 00	52	10 50	50	17 00	50	13 30	49
t for 48 Ex from graph	0 50		1 25		3 00		4 50		7 25	
1/t	2 0		0 8		0 333		0 222		0 137	
a in m moles	0 025		0 025		0 025		0 025		0 025	
1/c-a	33 38		40 00		45 66		51 81		83 69	

a=0 025 m moles n=0 098

TABLE 4

Coagulation of Au sol with KCl
Concentration of the sol—0·040 gms of $AuCl_3$ per litre.

Conc. of KCl in m moles →	30 0		29 0		28 0		27 0		26 0		25 0	
	Time	Ex	Time	Ex	Time	Ex	Time	Ex	Time	Ex	Time	Ex
	0 5	26	0 5	25	2 25	26	3 25	26	2 25	25	3 5	25
	1 25	27	1 25	26	4 25	27	6 50	27	3 75	26	8 25	26
	2 5	28	2 25	27	7 75	28	11 25	28	10 75	27	26 00	27
	4 0	29	5 00	28	12 00	29	16 5	29	19 00	28	31 5	28
	6 0	30	8 00	29	19 00	30	24 0	30	34 5	29	54 5	29
			11 25	30								
t for 28 Ex from graph	2 5 *		5 0		7 75		11 25		19 00		31 5	
1/t	0 400		0 200		0 129		0 088		0 052		0 031	
a in m moles	22 5		22.5		22 5		22 5		22 5		22 5	
1/x-a	0 133		0 153		0 181		0 222		0 285		0 400	

m = 5 09 m moles. n = 21 9

TABLE 5

Coagulation of Au Sol with $BaCl_2$

Concentration of the sol—0 040 gm of $AuCl_3$ per litre

Conc of $BaCl_2$ in m moles →	1 000		0 975		0 950		0 925		0 900		0 875	
	Time	Ex	Tim	Ex	Time	Ex	Time	Ex.	Time	Ex	Time	Ex
	0 50	24	1 75	24	2 25	23	2 50	22	3 25	22	5 25	22
	0 75	26	2 50	26	4 00	25	5 25	24	5 00	23	8 25	23
	1 00	28	3 75	28	5 00	26	7 00	25	7 00	24	12 00	24
	1 50	30	4 50	29	7 00	28	10 50	27	12 25	26	16 50	25
	2 50	32	5 00	30	10 00	30	12 25	28	21 00	28	21 50	26
	3 75	33	6 00	31	11 50	31	15 75	29	27 00	29	28 25	28
	5 00	34	8 00	33	13 50	32	19 75	30	34 00	30	36 5	29
			10 00	34	16 00	33	24 50	31			63 0	30
t for 28 Ex from graph	1 00		3 75		7 00		12 75		21 00		28 25	
1/t	1 00		0 268		0 142		0 078		0 047		0 035	
ln m moles	0 8125		0 8125		0 8125		0 8125		0 8125		0 8125	
1/c-a	5 333		6.153		7 272		8 888		11 428		16 000	

a=0 020 m moles n=0 151

TABLE 6

Coagulation of Au sol with $AlCl_3$
Concentration of the sol—0 040 gms. of $AuCl_3$ per litre

Conc of $AlCl_3$ ⟶ in m moles	0 0687		0 0656		0 0625		0 0594		0 0563		0 0532	
	Time	Ex	Time	Ex	Time	Ex	Time	Ex	Time	Ex	Tim	Ex
	1 00	28	1 73	27	2 50	26	1 75	25	3 25	25	5 00	25
	1 50	29	2 50	28	4 25	27	8 00	26	8 00	26	12 00	26
	2 50	30	3 75	29	8 50	28	7 5	27	14 00	27	.2 00	27
	2 50	30	5 00	30	9 00	29	12 00	28	22 50	28	41 00	28
					12 00	30	17 00	29	32 50	29	58 50	29
							25 00	30	39 00	30		
t for 28 Ex from graph	1 00		2 50		6 50		12 00		22 50		41 00	
1/t	1 000		0 400		0 153		0 083		0 044		0 024	
ln m moles	0 033		0 033		0 033		0 033		0 033		0 033	
1/ -n	28 3		31 3		31 7		38 9		44 2		51 2	

m=0 035 m moles n=0 037

Time of coagulation by light extinction was determined by a photoelectric colorimeter (Eel) Blue filter was used for Sb_2S_3 sol and a red one for the gold sol. The pointer of the apparatus was adjusted by taking distilled water in the tube to zero of the scale with the help of a screw attached in it. A mixture of water electrolyte and sol was taken in the same tube and light extinction was noted with time. The values of extinction (ϕ) were plotted against time and the same stage of coagulation was determined by drawing a line parallel to the time axis at a suitable value of ϕ to cut the $\phi-t$ curves c was then plotted against 1/t to determine a, 1/c-a was then plotted against the respective times of coagulation at that particular stage.

Discussion

The constants a, m and n of the equation $c = a + \frac{m \; 1/t}{n + 1/t}$ have already been interpreted in our previous communications (2) where a has been defined as the critical stability concentration of the electrolyte for the sol by which the coagulation does not take place within measurable time. 'm' is that concentration of the electrolyte which is required to coagulate the sol immediately For 'n' we have to proceed in a different manner Considering the dimensions of the terms in the equation,

$$c = a + \frac{m}{nt+1}$$

the factor nt+1 should have the dimension of concentration or it should have no dimension. Two possibilities thus arise either 'n' should have the dimension of T^{-1} or nt may have the dimension of M/l^3 Then n should have the dimension of M/l^3 1/t. If the latter dimension is considered, 'n' can be regarded as a function of concentration of the electrolyte divided by t Thus it can be connected with the concentration of the electrolyte required to coagulate the sol in unit time. Hence the value of n with increasing valency of the electrolyte should decrease, which is also supported by our observations.

In table no 1 to 6 the values of a, m and n have been recorded for the electrolytes KCl, $BaCl_2$ and $AlCl_3$ which are in the increasing order to valency of the precipitating ion

The value of 'a' which has been defined as the critical stability concentration of the electrolyte for the sol, remains the same for varying concentrations of the electrolyte. This had also been observed in the slow coagulation of As_2S_3 $Cr(OH)_3$ $Fe(OH)_3$, prussian blue and copper ferrocyanide by Bhattacharya and coworkers (loc cit.) The value of 'a' for the mono bi and tervalent precipitating ions are also in accordance with the Schulze hardy rule.

The value of 'm' are in the decreasing order with the increasing valency of the precipitating ions. The value of n for uni bi and tervalent electrolyte

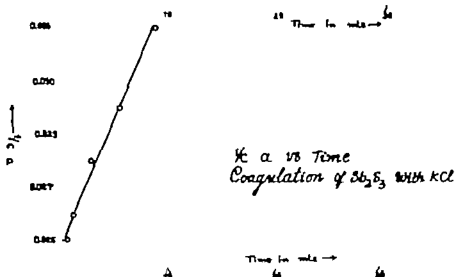
are also in the decreasing order. These observations testify to the merit of the equation $c = a + \frac{m \cdot 1/t}{n + 1/t}$ for the relation between concentration of the electrolyte and time of slow coagulation by simple techniques. Further work is in progress.

References

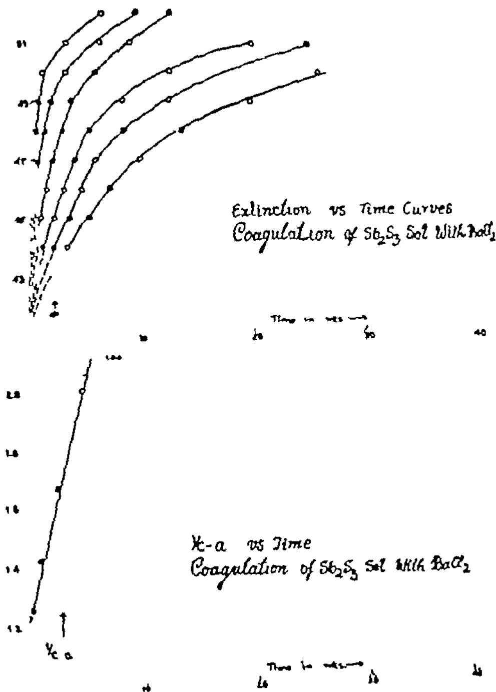
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54
52
50
48
46
44

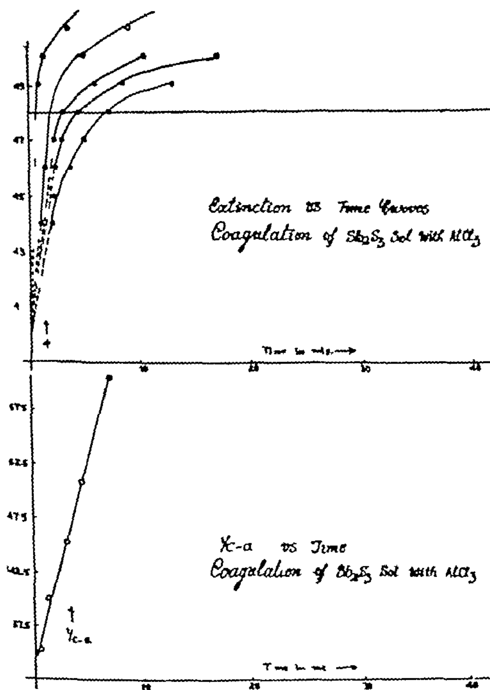
Extinction vs Time Curves
Coagulation of Sb_2S_3 Sol
With KCl



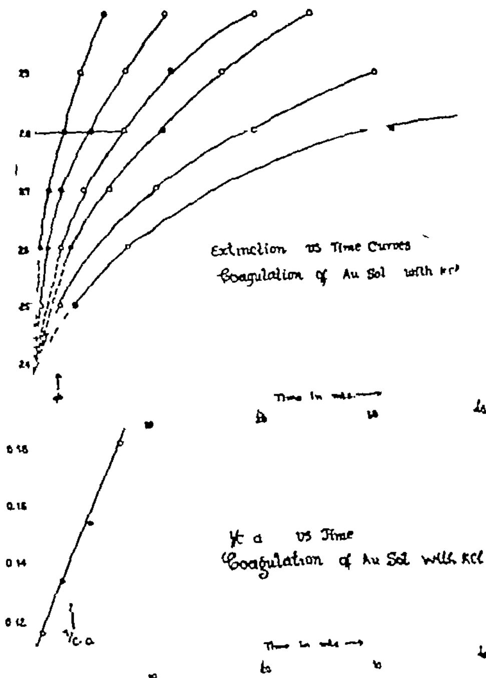
Graph No. 1


Extinction vs Time Curves
Coagulation of Sb_2S_3 Sol with $BaCl_2$
Time in mts →
10
20
30
40
1.8
1.6
1.4
1.2
$1/c$-a vs Time
Coagulation of Sb_2S_3 Sol With $BaCl_2$
Time in mts →

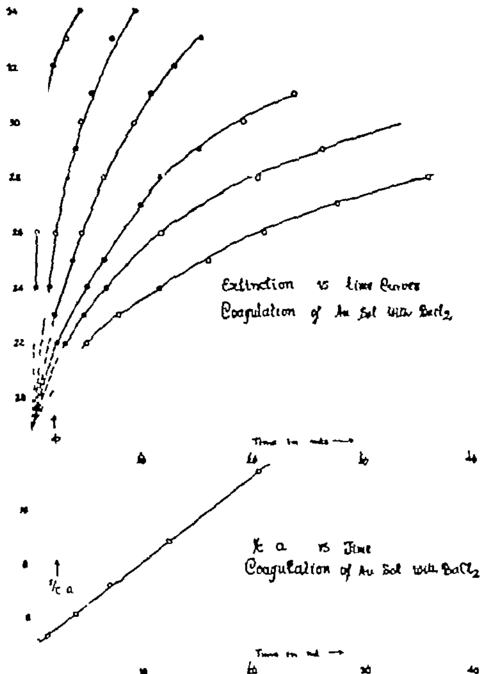
Graph No. 2.


Extinction vs Time Curves
Coagulation of Sb_2S_3 Sol with $AlCl_3$
Time in mts. →
$1/c-a$ vs Time
Coagulation of Sb_2S_3 Sol with $AlCl_3$
Time in mts →

Graph No 3


Extinction vs Time Curves
Coagulation of Au Sol with KCl
Time in mts
1/C a vs Time
Coagulation of Au Sol with KCl
1/C a
Time in mts

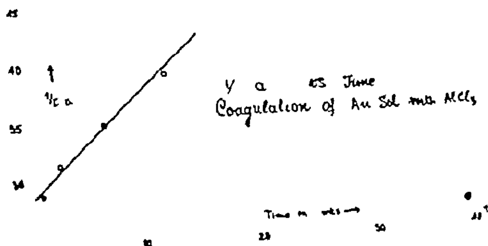
Graph No 4


Extinction vs time Curves
Coagulation of Au Sol with BaCl2
Time in mts →
1/C a vs Time
Coagulation of Au Sol with BaCl2
1/C a
Time in mts →

Graph No 5

ε Extinction vs Time Curves
Coagulation of Au Sol with $AlCl_3$

Time in mts →



Graph No. 6

OPTICAL ACTIVITY AND CHEMICAL CONSTITUTION

PART—IV OPTICALLY ACTIVE FORMS AND RACEMIC MODIFICATION

O N PERTI

Professor of Chemistry Th. D S B Government College Naini Tal

According to Pasteur's principle of Molecular Dissymmetry (Pasteur 1884) the *dextro* and *laevo* forms must possess the same total energy. They must also possess the same scalar properties such as density, viscosity, crystal lustre, solubility and so on. They must however differ in such vectorial properties as the direction of the rotation of the plane of polarization of light or un symmetrical distribution of hemihedral facets in the crystal forms in which these facets are developed (Frankland, 1897) or in the enantiomorphous dis tribution of pyro- and piezo-electrical polarity. The magnitude of these vecto- rial properties is identical for the enantiomorphous forms and the difference lies in the sign or direction. For instance in respect of their optical rotatory power each isomer rotates the plane of polarised light exactly the same number of degrees but in opposite direction

The enantiomorphs or optical antipodes contain the same number of atoms and groups in the same relative relationship to each other. Their chemical reactions are identical. The only differenc in arrangement of groups is a different spatial order—clockwise or anti-clockwise. In these reactions where the *dextro* and *laevo* forms of an active compound react with another optically active molecule the type of reaction would be identical though the rate of reaction may be different

On the basis of wave mechanics Temple (1930) suggested that the *dextro* and *laevo* form of a compound differ in energy and rotatory power. Campbell (1930 1931) reported slight but distinct differences in the rotatory power and other physical properties of the *dextro* and *laevo* forms of mandelic and camphoric acids in support of Temple's view and thus disputed the validity of Pasteur's principle of Molecular Dissymmetry. Kortum (1931) studied the case of *dextro* and *laevo* mandelic acid and showed that if the preparations are sufficiently purified, there are no measurable differences in the rotatory power of the optically active and opposite forms. Singh and Mahanti (Singh *et al.*, 1935a b) examined the case of (+) and (—)-camphoric acid. They employed the same process of purification as advocated by Campbell and found no difference in the magnitude of rotatory power in the enantiomorphs throughout the visible range of the spectrum (4358 to 6709 A. U). Inspite of these and other experimental evidences Bowden in 1938 wrote 'The supposed identity of the d and l forms is really an unproved assumption and, quoted Campbell's paper in support of the statement. B.K. Singh *et. al*

have studied several physical properties of the *dextro* and *laevo* forms with a view to experimentally examine the validity of Pasteur's principle of molecular Dissymmetry The physical properties such as density viscosity and refractivity of *dextro* and *laevo* forms of nonitroso camphors (stable and unstable forms) camphor camphoric acid, camphoric anhydride camphorquinone and sodium camphorate did not show differences beyond experimental error (Singh *et al* 1937) Raman spectra of the *dextro* and *laevo* borneol (Singh *et. al.*, 1937[c]) or camphoric acid or camphoric anhydride (Singh *et al* 1937[b]) did not show any results which were in contradiction with Pasteur's view The magnetic susceptibility measurements of *dextro* and *laevo* forms of camphor borneol and camphorquinone did not show any differences in optical antipodes (Singh *et al* 1944[a]) Similar results were found in the case of camphoric acid, camphoric anhydride and camphor-β sulphonic acid (Singh *et al.*, 1944[b]) nor was any discrepancy noted in the case of (+) and (—)-camphor-β-sulphonates of nitrogen bases (Singh *et al* 1949[a]) The ultraviolet absorption of the *dextro* and *laevo* forms is always found to be identical and so is found the magnitude of optical rotatory power The slight differences in physical properties wherever noticed are well within the limits of experimental error or else are due to a deficiency in the purity of the products under examination.

Whereas the physical properties of the *dextro* and *laevo* forms are identical in magnitude their physiological effects may or may not be identical. For a long time it has been known that the *dextro* and *laevo* forms may exhibit different physiological action. Pasteur in 1860 observed that the green mould *penicillium glaucum* destroys exclusively the *dextro* form in a solution of ammonium racemate containing a little potassium phosphate Several examples of this type are known and are listed in their papers by Winther(1895) McKenzie and Harden (1903) and in Werner's *Lehrbuch der Stereochemie* p 63 Frankland and Mac Gregor (1893) have recorded a curious observation that although fresh cultures of *bacillus ethaceticus* act only on the *dextro* salt of glyceric acid they can, by cultivation in a solution of calcium glycerate, be gradually induced to assimilate the *laevo* enantiomorph as well. This power of selective assimilation in living organisms finds a parallel in the different physiological action of *dextro* and *laevo* forms on the animal body and of the animal body on them. As examples of such differences may be mentioned the taste odour and toxicity Piutti (1886) found that (+)-asparagine has a sweet taste whereas the naturally occurring (—) asparagine is insipid Similar differences between (+) and (—) leucic acid and (+)-and (—)-glutamic acid have been observed-the (+)—form being sweet and the (—) tasteless (Kodama, 1920) v Braun and Kaiser (1923 1926) observed in certain cases differences in odour in *dextro* *laevo* and inactive forms. Singh and Lal (Singh *et al* 1939 1940) observed such differences in the *dextro* *laevo* and inactive forms of certain derivatives of amino- and bis aminomethylene camphors. They noticed that the intensity of the odour is greater in the *laevo* form than in the *dextro* form. Pictet and Rotschy (1901) found that

(−)-nicotine is for more poisonous than the (+) isomer Abderhalden (1909) noticed that (−) adrenaline is far more physiologically active than the (+)-isomer Chabrie (1893) showed that the *laevo* tartaric acid when administered to guinea pigs is twice as poisonous as the *dextro* form. Cushny (1916, 1921) found that the action of (−) hyoscyamin is hundred times the action of the (+)-enantiomorph. Demole (1934) has found that the dextro rotatory ascorbic acid is active but its antipode is inactive Singh *et al* (1944*) have noticed differences in the physiological action of *dextro laevo* and *racemic* forms of camphor β-sulphonates of some organic bases.

Like enzymes animal body can selectively assimilate and excrete certain isomers. (−)-Tyrosine for example, is more easily destroyed in the animal body than the (+)-isomer (Dakin, 1910 Pyman, 1917) *racemic* mandelic acid when injected subcutaneously in rabbit gives rise to (+) malate in the urine (Tomita 1921) and, when sodium-DL-phenyl-γ-oxybutyrate is given to a dog, the urine contains more of (−) than the (+) isomer (Thierfelder and Schempp 1917) It has also been found that certain optically active dyestuffs are selectively adsorbed by animal fibres such as wool (Ingersoll and Adams 1920 1926 Morgan and Skinner 1925) Porter and Thrigg (1933) could actually effect the resolution of *racemic* dyestuff by differential adsorption of one of the isomers by wool

It may be mentioned here that several views have been advanced to explain the different physiological activities of optically active isomers. Pasteur (1886) suggested that the nerve substances and the tissue substances are themselves asymmetric and therefore react differently towards *dextro* and *laevo* rotatory forms of physiologically active isomers King (1924) was of the view that the differences in physiological action of optically active isomers are chiefly due to physical rather than chemical causes Erlenmeyer (1919) advanced the hypothesis that the optical isomerides differ in physiological action because of 'asymmetric affin ty' which favours the action of on isomer in preference to its optical antipode. Cushny n his book *Biological Relation of Optically Active Substances* has reviewed the problem and remarks here some chemical combination occurs of such a nature that the isomers no longer form mirror images and these therefore differ in physical properties and their reactions. Easson and Stedmann (1933) have suggested another explanation According to them there is no essential difference in the causes which are responsible for the differene in physiological activity of the structural isomers on the one hand and those which are responsible for different physiological activity of the optical isomers on the other hand. These authors suggest that the differences are due to the facet of the tetrahedra contain ng different groups which come into contact with the specifically active receptors. Singh and Lal (Singh *et al* 1940) have reviewed these different theories and have pointed out that the theory of Easson and Stedmann is very much like the Werner s explanation of Walden Inversion in that tl ey both offer an equally insufficient explanation

of the phenomenon concerned. None of them can predict the course of action which a particular isomer would take.

Whereas the physical properties of the *dextro* and *laevo* forms are identical in magnitude, the properties of the DL- or *racemic* form are *sometimes markedly* different. Active isomers exhibit varying degree of stability and by suitable treatment most of them can ultimately be converted into inactive *racemic* modification. This process is termed *racemization* and *is brought about* by the conversion of one half of the active material into its enantiomorph. Racemization can occur by purely physical agencies such as heat light or solution in a solvent. It may occur *spontaneously at* room temperature when it is called *auto-racemization*. It may also be accomplished by change in structure. Compounds which have reactive functional groups attached to the asymmetric carbon atom undergo *racemization comparatively* easily and if the asymmetric carbon atom is in α-position to a group capable of undergoing a tautomeric change, racemization readily takes place. In all cases *racemic* modification can always be *obtained* by *mixing* equimolecular quantities *of dextro* and *laevo* forms.

The physical properties of a *racemic* modification generally differ from those of the active forms from which it is derived. It may exist in three forms in the solid state namely a mixture a compound or a solid solution (*pseudo racemic mixed crystals* a term used by Kipping and Pope) of the active forms. The *racemic* modification may be a mechanical mixture of the *dextro* and *laevo* forms and in some cases such as that of sodium-ammonium tartrate the crystals may possess hemihedral facets and be themselves enantiomorphic. *The dextro and laevo forms may* unite to form a molecular compound, all crystals of which contain equal amounts of both the active forms and are identical. In such a case the physical properties of the *racemic* modification are *generally markedly different from those of its* components. Ordinary cryoscopic methods fail to establish a multiple molecular weight of such a compound as *in solution* they almost completely dissociate into active forms (Raoult, 1887 Anschutz, *1888 Frankland and Pickard 1896 Bruni and Padoa*, 1902). If excess of one of the modifications is present the extent of dissociation *may* however be limited (Walden 1906 Patterson 1905). A solid *solution or a* pseudo racemic mixed crystal *is obtained if* the pair of enantiomorphs is also isomorphous. In such a case they may crystalise together as a *solid* solution without forming a compound. This solid solution differs from a mixture in that it constitutes but a *single* phase. *It differs from* a racemic compound in that all mixtures composed of a racemic solid solution and either active form still constitute or act as a *single* phase whereas the corresponding mixture composed of a racemic *compound and* either active form constitutes two phases.

The problem of distinguishing between these three forms of racemic modification was first discussed systematically by Bakhuis Roozeboom (1899, Jaeger 1917) from the point of view of phase rule. Roozeboom described ...

methods to distinguish between the three types of *racemic* modification viz. the Freezing Point Method and the Solubility Method

Freezing-Point Method—This method depends upon the principle that the presence of a second fusible solid phase lowers the freezing-point (or melting point) of an organic compound According to Roozeboom this method requires the preparation of a freezing point-composition (or melting-point composition) diagram for mixtures of *racemic* modification with its corresponding active isomers Then the distinction between three types of *racemic* modification can be made thus the diagram for a *racemic* mixture would be composed of two curves (Fig 1) for a *racemic* compound of three curves (Fig. 2) and for a *racemic* solid solution one curve (Fig 3) The three cases are briefly discussed below —

(i) Case—I *Freezing-point (or melting-point)-composition curve of a racemic mixture*

As the two enantiomorphs do not combine they may be considered as distinct entities. Therefore the addition of successive small quantities of one enantiomorph will lower the freezing-point (or melting-point) of the other until an equal quantity of each is present When the quantity of the second enantiomorph exceeds that of the first the reverse change will occur and the freezing-point (or melting point) will rise until the original freezing point (or melting-point) is reached In other words the curve would be of the type represented by acb in Fig 1 where the point c corresponds to equal quantities of the two enantiomorphs or the inactive mixture and, is known as the eutectic point.

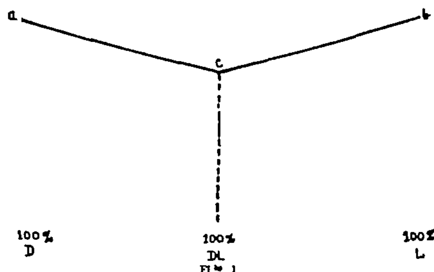


Fig 1

(ii) Case—II *Freezing point (or melting-point)-composition curve of a racemic compound*

In this case three entities are concerned namely the *dextro* and the *laevo* forms which possess the same, and the *racemic* compound which has usually a different (higher or lower) freezing-point (or melting-point) It is therefore convenient to consider the curve in two parts viz. the *freezing-point (or melting-point)*-composition curve of the *dextro* and the *racemic* modification, and, the *laevo* and the *racemic* modification. As the two active forms behave identically in the phenomenon under consideration each of these curves would be *mirror* image of the other Now each half of the curve can be compared to a simple mixture of two substances and the arguments given under case-I can be similarly employed. The curve in general would follow the route indicated by acbc'a in Fig 2 The point b would be in the same line as a or a or lower or higher according as the freezing-point (or melting-point) of the *racemic* compound is equal lower or higher than that of either active form.

Fig. 2

(iii) Case—III *Freezing-point (or melting-point)-composition curve of a racemic solid solution.*

It is possible that the two enantiomorphs form neither a mixture nor a compound but are isomorphous and form mixed crystals. The effect on the freezing point (or melting-point) of the mixture produced by the addition of either active form will depend upon the nature of the mixed crystal. If the freezing point (or melting poing) of the mixed crystal is the same as that of either active form then the curve would be a straight horizontal line joining the identical freezing-points (or melting points) of the enantiomers (acb in Fig 3) If on the other hand, the mixed crystals which separate from the fused mixture of the two active forms have a higher or lower freezing-point

(or melting-point) than either active form the curve would be of the type represented by ac'b or ac"b in Fig 3

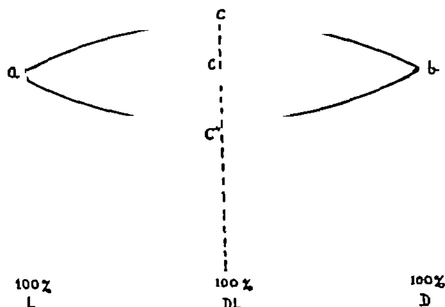


Fig. 3

Examples of all the three types of curves are known (Markwald and Nolda 1909) Bruni (1899) has suggested a modification to Roozeboom's freezing-point method. This modification involves the study of the eutectic mixtures of the two active forms with a third *inert* substance. Adriani (1901) studied camphoroxime by this method. The *inert* substances used by him were naphthalene, phenanthrene benzoin and anthracene. His curves indicated that between 60° and 90° *racemic* camphoroxime exists as a compound but at 106° as a solid solution.

This method of Roozeboom gives results wh ch are valid only at about the temperature of fusion. Consider the case of a *racemic* compound where the freezing-point (or melting point)-composition diagram (Fig 2) consists of three curves. Now suppose that at the temperature of fusion the *racemic* compound is dissociated into *dextro* and *laevo* forms. It is obvious that the greater the dissociation the less would be the lowering of the freezing point (or melting point) when either *dextro* or *laevo* is added to the *racemic* compound. It follows that the greater the dissociation, the flatter would be the freezing-point (or melting point)-composition curve. In other words the flatness of the curve would depend upon the stability of the *racemic* compound at the temperature of fusion.

Singh and Perti (Singh *et al.* 1943 1944*) have studied the melting point-composition curves of *racemic* and active modifications of camphorquinone, camporic acid, camphoric anhydride, camphor-β-sulphonic acid and

its salts with aniline, toluidine (o- m- and p-) napthylamine (α β-), and ar tetrahydro-α naphthylamine. In all cases *they found that the racemic* modification is a true compound at the temperature of fusion and is fairly stable in all cases except that of camphoric anhydride Singh and Tewari (Singh *et al.*, 1947b) studied the nature of *racemic* modification with the help of melting-point-composition curves in the case of 3-nitro-p-toluidino-5-iodo-o-toluidino- 4-nitro-o-toluidino- oxy- 2 nitro-p-toluidino- 3-nitro-4-chloranilino- 2.5-dichloroanilino-and 4-chloro-o-toluidino-methylene camphors. They found that the *racemic* modification in each case is a compound except in the case of 3 nitro-p-toluidino-methylene camphor which is a mixture at the temperature of fusion. From the nature of the curves obtained they also discussed the stability of various *racemic* compounds. Singh and Nayar (Singh *et. al.*, 1949b) studied the degree of dissociation of *racemic* modification of camphor carboxylic acid at its melting point. Singh, Panicker *and Ranganathan (Singh et al., 1952)* applied Roozeboom's melting-point method to find the nature of *racemic* modification in the case of camphor-β-*sulphonamide, camphor β-sulpho anhydramide* camphor *β-sulpho-phenyl-*, o-tolyl, m-tolyl p-tolyl α naphthyl-and β-naphthyl-amides and noted that all the eight *racemic modifications studied were true compounds of dextro* and *laevo* forms. Singh and Miss Amma (Singh *et. al.* 1953) carried out similar studies in the case of o- m- and p-chloranilino-camphor-β-sulphonates and camphor-β-sulphonyl-o- m- and p-chlorophenylamides. These workers tried to develop an equation for the freezing point curves but found that the *systems* represented by the experimental curves are complicated and their actual behaviour is not sufficiently understood Singh and Miss Seth (Singh *et al* 1956a) studied the nature of *racemic* forms of phenyl o m-, p-chlorophenylimino camphors and phenyl, o- m-, p-chlorophenylamino comphors and found that the *racemic* forms of o-chlorophenylimino and aminocamphors are solid solution whereas the remaining forms are true compounds. m-*Chlorophenylamino-(±)-camphor* exists in dimorphic forms (α and β-) Study of the melting-point-composition curves by these authors suggested a *new* method for the determination of transformation (transition) temperature of the dimorphic forms. The method is *If the transition temperature* is above the melting-points of the mixtures of β-form with a third substance *(d form)* the curve of the metastable form (α) will always be above that of the stable form (β). If however the transformation temperature is below the melting-points of the mixtures, the melting point curves of α form and the β-form with *d form* would be identical. The point where the two curves meet will be the transformation temperature. The temperature of transformation of α-form into β-form in the case of *racemic* form of m-chlorophenylamino camphor was *found to be* 116 1° Singh and Miss Amma (Singh *et al.*, 1955b) in the case of *ami*-forms of camphor β-sulphonyl-o- m- and p- *bromophenylamides* and o- m- and p- bromanilino-camphor β-sulphonates found that the *racemic* camphor forms are true compounds It was also observed *that the racemic* camphor

β-sulphonyl-o- m- and p-bromophenylamides have a very low range of stability and form solid solutions between the composition ranges 10 *dextro*:90 *racemic* to about 60 *dextro* 40 *racemic* The o- m and p-bromanilinocamphor -β-sulphonates were however found to be quite stable. Singh and Verma (Singh *et. al.*, 1957[a]) examined the nature of *racemic* modification of camphor-β-sulphonyl-o- m and p-nitrophenylamides camphor β-sulphonyl o- m- and p-phenylene diamines and o- m and p-phenylene-*bis*-camphor β-sulphonylamides and found that camphor β-sulphonyl-m and p-nitrophenyl amides camphor-β-sulphonyl-p-phenylene diamine and o-phenylene-*bis* camphor β-sulphonylamide exist as solid solution and the rest are true compounds of the *dextro* and *laevo* forms. Singh and Sarma (Singh *et. al.*, 1958) studied the nature of *racemic* modifications of methyl ethyl, phenyl o- and p-tolyl and α- and β-naphthyl-camphor β-sulphonates. They observed that the *racemic* modifications of these esters are true compounds except in the case of β naphthyl-camphor β-sulphonate which exists as a solid solution. Singh and Verma (Singh *et al.* 1957[b]) found that the *racemic* forms of camphor β-sulphonyl-o- m- and p-methoxy phenylamides and camphor β-sulphonyl-m and p-ethoxy phenylamides are true compounds of the *dextro* and *laevo* forms In these compounds, it was found, that the *racemic* modification of camphor β-sulphonyl -m-methoxy phenylamide exists in dimorphic forms (α and β-) and the transition temperature of α into β- form is found to be 92.7 92.8° Singh and Saxena (Singh *et al.*, 1958) observed that whereas the *racemic* forms of o- and m-ethoxyphenylaminocamphor exist as solid solution, those of p-sulphonamido- p-ethoxyphenylaminocamphors are true compounds. Later these workers (Singh *et. al.* 1960) studied the *racemic* forms of camphanoquinoxaline, camphanodihydroquinoxaline, m and p-phenylene-*bis*-imino and amino camphors by similar methods They found that out of these camphanoquinoxaline m-phenylene-*bis*-amino and imino-camphors exist as solid solution They also discussed the stability of these *racemic* forms with the aid of their melting-point-composition diagrams.

From the foregoing it is evident that one can easily distinguish between a *racemic* mixture and a *racemic* compound. The freezing point (or melting point) of the substance under consideration be determined and then a small amount of pure either active form be added to it and its freezing point (or melting-point) be determined again If the freezing-point (or melting point) is higher it is a mixture and if lower it is a compound. The distinction between a compound and a solid solution can only be made if the freezing point (or melting-point)-composition curve of the *racemic* and either active form is traced.

Solubility Method—Roozeboom pointed out that just as the presence of a second fusible phase lowers the freezing point (or melting point) of an organic compound so the presence of a second soluble phase alters its solubility Therefore, if a solubility-composition isotherm for mixtures of a *racemic* modifica

tion and its corresponding active forms is plotted, a *racemic* mixture gives rise *to two curves*, a *racemic* compound to three curves and a *racemic* solid solution to one curve. Any conclusions drawn from such mixed solubility determinations are only valid for the particular temperature at which the solubilities are determined

(i) Case—I *Solubility composition curve of a racemic mixture*

The general shape of the curve would be shown as in Fig 4 The actual shape of the curve would depend on the nature of the solute and the solvent. The point c may lie on a straight line joining a and b or may be below or above this line.

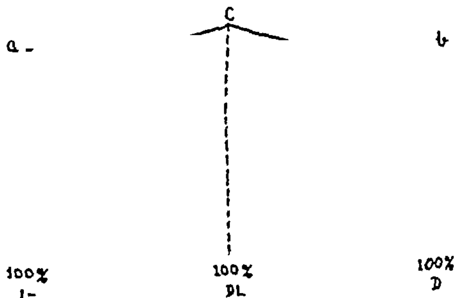


Fig 4

(ii) Case—II *Solubility composition curve of a racemic compound.*

The general shape of the curve would be like acbc a in Fig 5. Since *dextro* and *laevo* forms behave identically in the phenomenon under consideration the curve would be symmetrical about the central dotted line The curve in general would follow the route acbc a The point b may be in a line with aa or lower or higher according as the solubility of the *racemic* form is equal to or lower or higher than the solubility of the active forms.

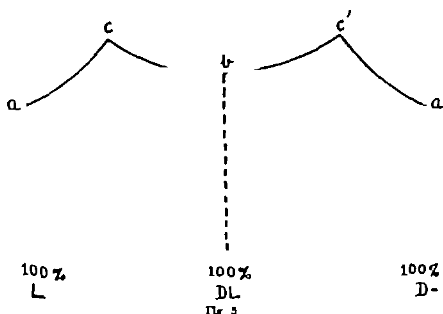


Fig 5

(iii) Case—III *Solubility composition curve of a racemic solid solution*

Solid solution differs from the mixture in constituting a single phase Hence in this case only one curve shall be obtained. The general shape of the curve would be ac′b, acb or ac b as shown in Fig 6 The position of the middle point c, c′ or c depends upon the solubility of the mixed crystal as compared to the solubility of either active form.

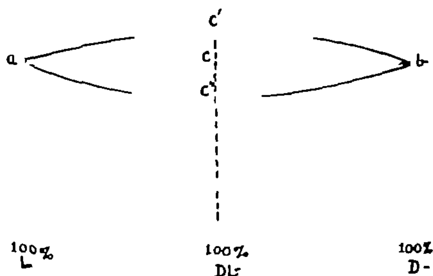


Fig. 6

It may again be emphasized that the conclusions drawn from such determinations are only valid at the temperature at which the determinations are carried out. The same substance which behaves as a racemic mixture at one temperature may behave as a racemic compound at another temperature. A classical example is that of sodium ammonium racemate (tartrate) which below 27 7° behaves as a racemic mixture and above this temperature as a racemic compound.

The role of solvent is also important. Racemic modifications sometimes crystallise from one solvent as a mixture, from another as a compound. Probably the solvent of crystallization also plays an important role thus sodium ammonium tartrate as a mixture crystallises with four molecules of water for each active form but as *racemic* compound with only two rubidium tartrate crystallises as a *racemic* compound with two molecules of water but as a racemic mixture with none.

Singh and Nayar (Singh *et. al.*, 1945ª) studied the solubility-composition isotherm of active and inactive camphoric acids at 35° They found that the solubilities of the *dextro* and *laevo* forms are identical. The solubility-composition isotherm at 35° was found to consist of three curves indicating that the *racemic* modification is a true compound of the *dextro* and *laevo* forms Similar result was obtained by Singh and Perti (Singh *et al* 1945ᵇ) in the case of *racemic* camphor-sulphonic acid. Singh and Nayar (Singh *et al.*, 1947) later extended their work on *solubility-composition isotherm of* camphoric acid in water at 25° 45° and at the boiling point of the solutions. In each case they found the solubility-composition isotherm to consist of three curves indicating that the *racemic* form is a true compound at these temperatures. The results obtained by the solubility method were found to be in agreement with those obtained by Singh and Perti (Singh *et al.*, 1944) by the study of melting-point composition curves of *racemic* camphoric acid with either active form A correlation is possible in this case between the solubility curves and melting-point curves. The percentage composition at eutectic points as determined by Singh and Perti was 80 *dextro* 20 *racemic* This is quite in agreement with the value 88 *dextro* 12 *racemic* at 100° as determined by solubility method. It is interesting to note here that according to Ross and Somerville (1926) the latent heat of fusion for the active forms of camphoric acid is 4,833 cals. and for the *racemic* form 13,840 cals This great difference in the latent heats of the active and inactive forms points to the great stability of the *racemic* form even at its melting point. Singh and Nayar (Singh *et. al* 1949) have also studied the degree of dissociation of *racemic* camphor carboxylic acid at its melting-point by plotting the melting point-composition curves.

It is obvious from the above discussion that in order to distinguish between a *racemic* mixture and a *racemic* compound it is only necessary t add a crystal of the pure *dextro* or *laevo* form to a saturated solution of the *racemic* modification at the *same temperature* In case of *racemic* mixture the crystal

will not dissolve and the solution will remain optically inactive In the case of *racemic* compound the addition of crystals of either active isomer results in the presence of a new solid phase and the crystal dissolves making the supernatant liquid optically active This method cannot distinguish between a *racemic* mixture and a *racemic* solid solution

It may be mentioned here that on the basis of different physiological action of *dextro laevo* and *racemic* forms Singh *et. al*., (1936, 1914[d]) have suggested a Biochemical Method to distinguish between a *racemic* mixture and a *racemic* compound. The physiological action of the active isomers is generally markedly different but that of the *racemic* modification will depend upon whether it is an equimolecular mixture of the *dextro* and *laevo* forms or a compound If it is a mixture then its effect would be intermediate of the two components. If on the other hand, the *racemic* form is a compound then its effect may be the highest or the lowest of the three isomers This method for obvious reasons cannot distinguish between a mixture and a solution Singh and Miss Amma (Singh *et. al* 1957[a]) have also pointed out that in concentrated solution a *racemic* compound can give different ultraviolet absorption spectra which, however on dilution will become identical with that of either active form

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REDUCTION PROCESSES AT THE DROPPING MERCURY ELECTRODE IN PULSATING FIELDS—COPPER ION

H P AGARWAL AND S SINGH

Department of Chemistry Motilal Vigyan Mahavidyalaya Bhopal

INTRODUCTION

The alternating current polarography as developed by Breyer and Gutmann can suitably be employed for analysis of the mixture[1] containing two or more metallic ions, whose half wave potentials are quite close to each other and which cannot b analysed by th conventional direct current polarography Breyer Gutmann and Hacobian[2] have obtained interesting results while studying th effect of superimposed alternating current of 50 cycles per sec. on the discharge of copper bismuth antimony and tin (using supporting electrolyte containing halogen ions) at a dropping mercury electrode.

In the present work, the redu t on of copper ion at the dropping mercury electrode in the pulsating field using 0.5 M K_2SO_4 as supporting electrolyte has been studied. The frequency of the superimposed alternating current was varied from 50 cycles per sec to 1000 cycles per sec The disappearance[3,4] of the Breyer Gutmann a.c. maximum at higher frequency depends upon the rate of discharge of the ion and the external resistance used in the dropping mercury electrode circuit hence the effect of external resistance has also been studied. The pH of the medium influences[5] tremendously the half wave potential of the discharging ion, therefore a few observations were also recorded using buffers of different pH as the supporting electrolyte.

EXPERIMENTAL

The technique for measurement of a. c. component of the pulsating current as adopted by Doss and Kalyanasundaram was modified in two ways. Firstly the measurements were taken in the region where the amplification of the two stage I L D 5 amplifier may be linear at a particular frequency of alternating current. Secondly all through the measurements the external resistance in dropping mercury electrode circuit was kept constant lest its variation may not bring about the corresponding variation in the a.c. component measured. These things were achieved by using a circuit diagram as given in Fig 1 The a.c source used was a Phillips audio frequency oscillator (type G M. 2315) capable of giving frequencies from 20 cycles per second to 20000 cycles per second. The output from the oscillator was fed to a potentiometric arrangement consisting of resistances R_1 & R_2 through key K_D so that a.c. voltage of 15 mv (r.m.s.) was incident on the dropping

mercury electrode. The output from the oscillator could also be directly connected to the variable resistance R_4 through key K_1. On pressing the switch S_1 an a.c. voltage of low magnitude was directly fed to the input of two stages I L D 5 amplifier A whose output was subsequently rectified with a selenium rectifier R_9 and the d.c. produced was measured by observing the deflection in the galvanometer scale. The magnitude of a.c. voltage was measured by connecting the end B of resistance R_4 to Y axis of the Philips cathode ray oscillograph (GM 3156 sensitivity 1 mv r.m.s./cm) through key P_2. Before taking any measurement of rectified d.c. produced due to the a.c. input given, the initial deflection obtained on the galvanometer scale when the a.c. input in the amplifier is zero (but for a slight a.c. hum) was balanced by using an opposing e.m.f. from the potentiometric arrangement provided by the resistance R_7 and cell B_2. The resistance R_8 served as a shunt to the galvanometer. Separate plots were obtained for each kind of a.c. frequency used (50 cycles/sec to 1000 cycles/sec) in between the a.c. input voltage (connected to the amplifier) and the deflection on the galvanometer scale obtained due to the rectified d.c. produced (keeping the galvanometer shunt constant). The observations were confined to the linear portion of the plot obtained above (for amplifier characteristic at particular frequency). Knowing the deflection on galvanometer scale the magnitude of a.c. at any frequency could be obtained from the respective plot.

A d.c. potential of known voltage is tapped from a Leeds and Northrup potentiometer P. The positive terminal of the output is connected to the pool electrode of the dropping mercury electrode assembly and the negative is being connected to the end B of variable resistance box R_4. The other end of the dropping mercury electrode assembly is connected to earth through the two resistance boxes R_5 and R_6. The a.c. component of the current (with the dropping mercury electrode assembly in the circuit) is measured across R_6. The magnitude of a.c. voltage incident across R_6 is adjusted by varying R_6 so that the observations at any frequency may be confined to range in which the amplifier characteristics are linear. All through the experiments resistance R_5 is so adjusted that the total of external resistance (R_5+R_6) in the dropping electrode circuit may remain constant. On pressing switch S_2 the pulsating field across R_6 is connected to the input of amplifier A through a condenser (so as to filter off d.c.). The a.c. voltage obtained from the output of the amplifier is rectified by the selenium rectifier R and a corresponding deflection is obtained on the galvanometer scale. Knowing the deflection, the magnitude of a.c. voltage incident across R_6 can be obtained from the amplifier characteristic plot at the respective frequency. On pressing key P_r the frequency of the a.c. output was checked from time to time.

The dropping mercury electrode has the following characteristics in open circuit —

m = 0.388 mg/sec.

t = 3.5 sec./drop in 0.5 K_2SO_4 solution

The experiment is first performed with 0.5 M aqueous solution of K_2SO_4 (A. R.) freed from air (by bubbling pure hydrogen) and containing no other dischargeable ions. The experiments are carried out at different frequencies of a. c. and are represented in Fig. 2. The experiment was repeated with 0.5 M K_2SO_4 solution containing 2×10^{-3}M copper ion (dissolved in the form of $CuSO_4.5H_2O$ A. R.) and the results obtained are given in Fig. 3.

The effect of external resistance on the Breyer and Gutmann a. c. maximum was studied at frequencies varying from 400 cycles to 1000 cycles per second and the results obtained at a frequency of 1000 cycles per second are given in Fig. 4.

Figs. 5 and 6 represent the results obtained with a buffer of pH 3.1 and with a citrate buffer of pH 6.9 containing 2×10^{-3}M copper ion (in the form of copper sulphate A. R.) respectively.

DISCUSSION

The behaviour of 0.5 M potassium sulphate at frequencies from 50 cycles per second to 600 cycles per second is shown in Fig. 2.

An examination of Fig. 2 shows that the a.c. current starts with a high value in the vicinity of .3 volts and then falls rapidly upto .5 volts. The fall of a. c. current in the above voltage range coincides approximately with the region in which the electro-capillary maximum occurs. The voltage of pool with reference to the saturated calomel electrode is 1.14 volts.

VARIATION OF ALTERNATING CURRENT WITH APPLIED D. C. VOLTAGE IN PRESENCE OF A REDUCIBLE ION IN SOLUTION—EFFECT OF FREQUENCY

In accordance with Breyer and Gutmann, it can be seen from Fig. 3 that the alternating current maximum is obtained at nearly the half wave potential of copper at all frequencies in between 50 cycles/sec. to 1000 cycles/sec. It is to be seen that the maximum goes on increasing with the increase in frequency even upto 1000 cycles/sec. when external resistance used in the circuit is 200 ohms. This can be explained from two points of view viz., the molecular kinetic and secondly the electrical equivalent of the dropping mercury electrode. The former point of view may first be discussed. At low d. c. voltages of the order of 0.1 volt there is no discharge of copper ions and the system behaves as a perfectly polarizable electrode. The capacitative impedance of the electrical double layer at the dropping mercury electrode is large, hence the resultant alternating current is small. The system becomes imperfectly polarizable due to the reduction of copper ions as the half-wave potential is reached. At the half wave potential the three important factors are to be considered viz. the electrode process, the diffusion process and thirdly the external resistance of the system. It may be noted that the diffusion processes are more or less similar in all the systems studied.

as the diffusion coefficients are of the same order of magnitude This would not, therefore bring about material differences in the behaviour of the different systems. If the external resistance is kept constant in different systems as well then the main cause of the difference in behaviour would appear to be due to the rate of electrode processes This may vary several thousand fold in different systems. Owing to the slowness of the electrode reaction, the electrode process is unable to keep pace with the a c. field at high frequency Therefore, with a slow reaction as in the case of discharge of nickel ions at the dropping mercury electrode, the maximum disappears away at 100 cycles/sec., whereas with copper (a faster reaction) it (maximum) is perceptible even at 600 cycles/sec. while using the external resistance of the same order (600 ohms) in both the circuits.

The results can also be explained in terms of electrical equivalent of the dropping mercury electrode. The large rise in alternating current at the half wave potential results due to the diminution in the impedance of the dropping electrode system.

Effect of External Resistance in the Dropping Electrode Circuit on A. C. Maximum

When using an external resistance of the order of 200 ohms it can be seen from Fig 3 that the a. c maximum is observed even upto 1000 cycles/sec. If the external resistance used in the circuit of dropping mercury electrode is of the order of 500 ohms the a. c maximum disappears away at 1000 cycles/sec. as is shown in Fig 4 Therefore, it may be concluded that the persistence of a. c maximum at any frequency will depend upon the magnitude of external resistance used in the dropping electrode circuit. With the variation in external resistance the half wave potential does not change (as can be seen from Figs. 3 and 4) showing thereby that iR drop is small. Similar results have been reported by Kalayansundaram[3] using lead ions in solution and by Agarwal[4] using zinc ions.

The disappearance of the a. c maximum at any frequency with the increase in external resistance of the circuit may be due to the over-all increase in the total impedance of the circuit including that of the electrical equivalent of the dropping mercury electrode

If the external resistance in the circuit is kept constant (say 200 ohm), the a. c. maximum diminishes with the lowering in frequency as shown in Fig 3 due to the increase of the capacitative impedance of the electrical double layer at the dropping mercury electrode (as the total impedance including the external resistance would increase which would in turn decrease the a. c maximum) The variation and the characteristics of the electrical double layer caused by the external resistance can be predicted theoretically on the basis of the iR drop in the absence of the transient effects of the type reported by R. Narayan V K. Venkatesan and K. S G Doss.[7] The quantitative

interpretation of resistance effect is somewhat complicated due to the following reasons —

(i) The electrolyte contains polar molecules, the dielectric constant under alternating current conditions, is a result of the rotation of polar molecules under the influence of the applied voltage. The extent to which the polar action is affected depends upon the frequency and the temperature. These effects[6] may also partially be responsible for altering the capacitance corresponding to the electrical double layer at the dropping mercury electrode in the absence of slow relaxation effect of the type envisaged by Narayan and coworkers[7].

(ii) The change in the growth of the mercury drop at the electrode under the varying influences is likely to affect the capacitative impedance of the electrical double layer.

EFFECT OF pH OF MEDIUM ON THE A. C. MAXIMUM

The pH of the medium seems to have a marked effect on the half wave potential of the reducible ion, as obtained from d. c. potential values at which the a. c. summit is observed. The half wave potential value at pH 3.1 is lower than that obtained at $pH^{5.2}$. At higher pH ($pH^{6.8}$) not only the magnitude of half wave potential increases further but instead of a single peak, two peaks are obtained in the curve (Figs. 5 & 6). The splitting up of the summit into doublet wave apparently has resulted due to the reduction of citrate complexes in sluggish equilibrium. This behaviour seems to be somewhat in analogy with the tartrate system, as has been studied by Meites[8]. At lower and higher frequency the general behaviour remains unaltered.

SUMMARY

The reduction of copper ions at the dropping mercury electrode has been studied in the superimposed alternating fields of varying frequencies. The summit current at all frequencies appears at the half wave potential upto 1000 cycles per second, when the external resistance of the cell circuit was 200 ohms. When the external resistance in the cell circuit is increased upto 500 ohms the a. c. peak disappears away even at 1000 cycles per second. The results have been explained on the basis of diminution in the over-all capacitative impedance of the dropping mercury electrode as the half wave potential is approached as well as when the frequency of the alternating field increases.

With the increase in pH of the media the half wave potentials are markedly influenced and at pH beyond seven the wave is found to be splitted up into a doublet due to copper citrate complexes.

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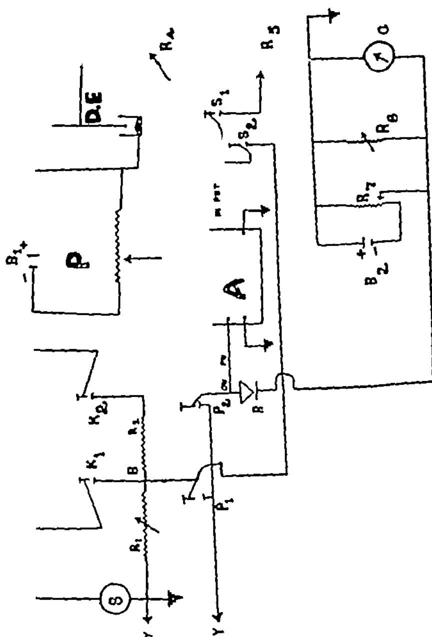
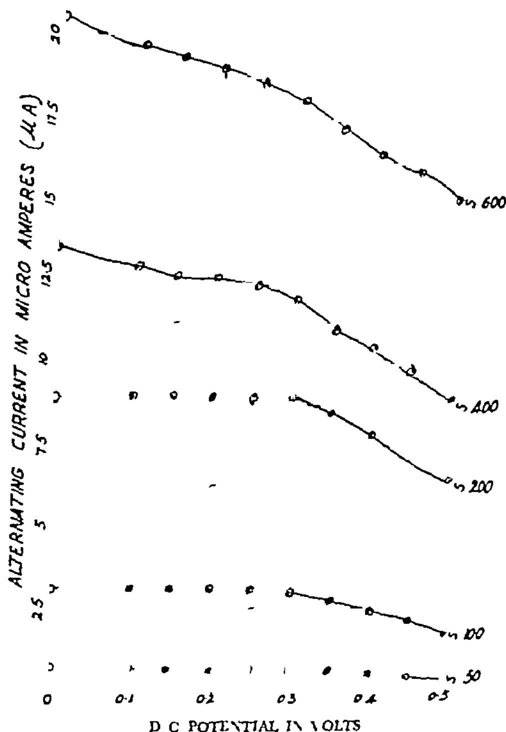
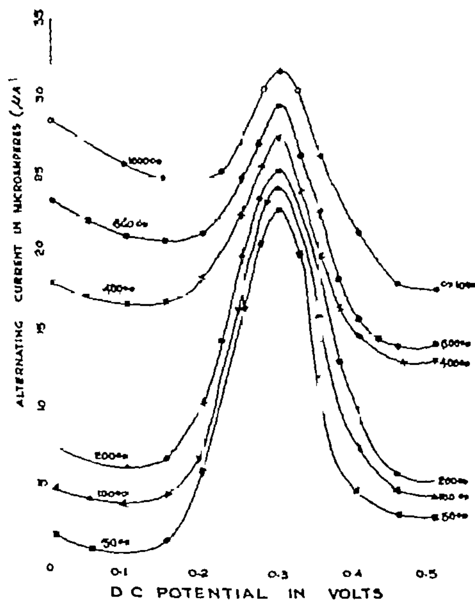


FIG 1

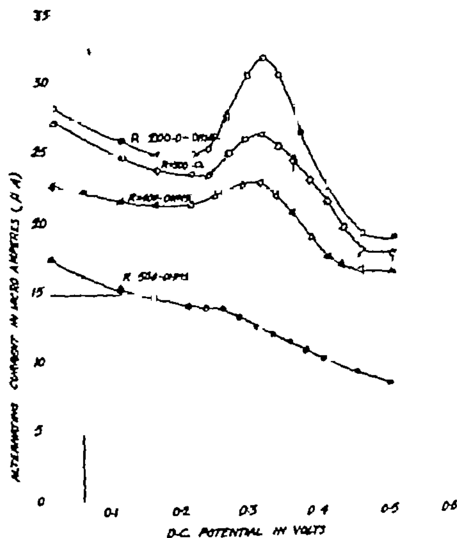


D C POTENTIAL IN VOLTS
Behaviour of Potassium Sulphate 0·5M in Pulsating Fields
Fig 2.



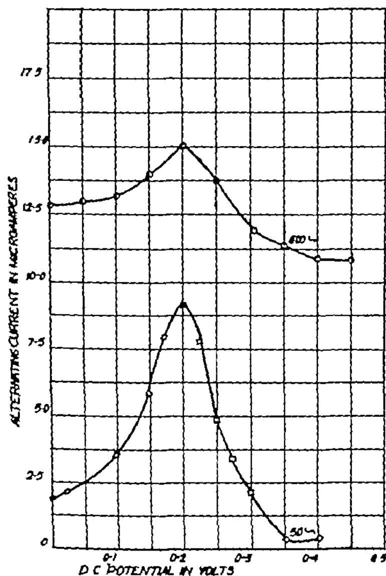
Effect of Frequency on Behaviour of Dropping Mercury Electrode (Copper Ion 2×10^{-4}M in 0.5 K_2SO_4) in Pulsating Fields.

Fig 3



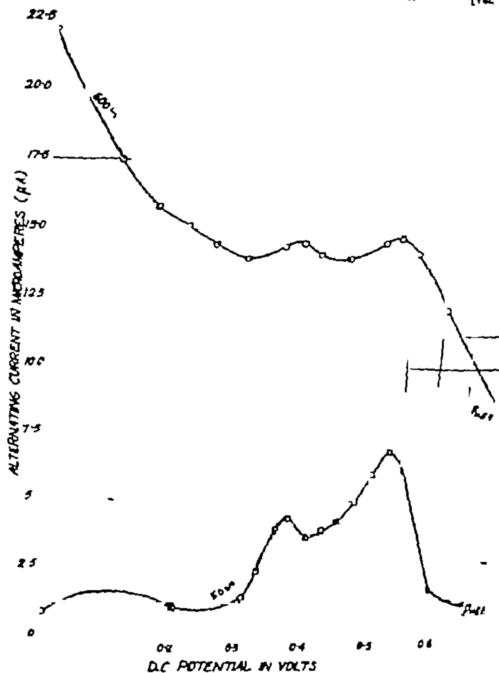
Effect of External Resistance in Cell Circuit on the Behaviour of Dropping Electrode (Copper Ion $2 \cdot 0 \times 10^{-3}$M in $0 \cdot 5$M K_2SO_4) in Pulsating Fields. Alternating Current Frequency 1000 Cycles/Sec

Fig 4



EFFECT OF P_H ON THE BEHAVIOUR OF THE DROPPING MERCURY ELECTRODE IN PULSATING FIELDS
COPPER IONS CONCENTRATION IN CITRATE BUFFER OF P_H ≈ 1 $2.0\ 10^{-3}$M

FIG. 5



EFFECT OF P_H ON THE BEHAVIOUR OF DROPPING MERCURY ELECTRODE IN PULSATING FIELDS
COPPER IONS CONCENTRATION IN CITRATE BUFFER OF P_H 6·0 2·0×10⁻³M

FIG 6

MORPHOLOGICAL STUDIES IN SOME MEMBERS OF THE FAMILY PEDALIACEAE—II *PEDALIUM MUREX* LINN

S P SINGH
Department of Botany B R College Agra.

Pedalium murex is a small xerophytic herb bearing yellow flowers and spinous fruits. There are two pink coloured nectaries on the sides of the pedical similar to those of *Sesamum indicum*. Srinivasan (1942) has described the embryology and Rao (1953) the floral anatomy of the plant Both of these works are fragmentary and at places the observations are inconclusive No one has studied the morphology of either the nectary or the spines of the fruit The present paper deals with the observations on embryology floral anatomy dev lopment of nectary and its anatomy seed and fruit structure. These observations will finally help in understanding the relationship of this genus with those like *Sesamum indicum* witl dehiscent non spinous fruits and *Martynia diandra* and *Trapella sinensis* with indehiscent spinous fruits.

MATERIAL AND METHODS

The material of *Pedalium murex* was collected from Banda Baretha, Bharatpur (Rajasthan) during the months of August and September and was fixed in formalin-acetic alcohol mixture Fixed flower buds, open flowers fruits and seeds were dehydrated and infilterated through ethyl alcohol xylol series and tertiary butyl alcohol series. The material was then embedded in paraffin and sections 8-20 μ thick were microtomed. The staining procedures for the sections and the whole mounts of pollen grains were the same as those followed for *S indicum* (Singh 1960)

OBSERVATIONS

Floral Morphology—The flowers are hypogynous, zygomorphic and pentamerous. The androecium, however consists of four stamens and a staminode. The stamens are ep petalous and didynamous with glandular protruded con nectives, the gynoecium is bicarpellary bilocular superior having two pen dulous, anatropous and unitegmic ovules in each loculus

Organogeny of flower—Th floral primordium arises as a dome shaped structure in the axil of a leafy bract (Fig 1) The calyx initials are first to arise (Fig 2) followed by corolla and stamens (Figs. 3 and 4) The central portion of the flower initial, left after this differentiation becomes broader and produces two semicircular outgrowths as carpellary initials (Figs 5 and 6) At the place of the fusion of the carpellary initials, placentae arise (Figs. 9 10) They grow inwards and ultimately meet in the centre dividing the ovary cavity into two chambers (Figs. 7 10 and 11) excepting in the upper

most region of the ovary The floral parts, therefore, are differentiated in acropetal order

Development of Nectary—Nectary also arises as a small protuberance in the axil of a bract like a flower (Fig 12) and gradually gets differentiated into a structure with four whorls of appendages *comparable to calyx* corolla androecium and gynoecium (Figs 13-18) When all the whorls of the appendages of the nectary are laid down the inner two whorls take up deep stain A similar behaviour of the inner whorls is also seen in *Sesamum indicum* (Singh 1960) and has been attributed to the taking up of the secretory function by these organs*

Vascular supply of flower—The node bears two lateral leaves, each of which receives a single leaf trace from the stem stele. This is soon followed by a pair of branch traces arising from the adjoining slides of each leaf gap (Fig 19) Each branch trace gives out a strand laterally to supply the nectary of its side (Figs. 20-22) The remaining tissue of the branch traces organizes itself into a more or less cylindrical structure and ascends in the pedicel of the flower (Fig 22) The bract subtending the nectary however does not receive any vascular supply (Fig 21)

Fig 23 represents the perspective view of the flower showing the levels from which the transverse sections have been drawn. Transverse section of the pedicel shows a complete ring of vascular tissue (Fig 24) and at a slightly higher level ten traces emerge from it (Fig 25) The five alternative ones of these traces enter the calyx as the midvein bundles (Fig 26 km) while the other five are the common traces for the calyx and the corolla. Each one of these traces separates into two calyx laterals and corolla trace (Fig 26, kl c)

The calyx laterals move out laterally and enter the adjoining sepals (Figs. 26-28) Each of the corolla traces, on entering the corolla first divides into three (Figs. 29 33) and then the resultant branches further subdivide as they ascend the corolla tube (Figs. 31 32)

From the main stele five more vascular traces are given out to the epipetalous stamens (Figs. 28-30) These bundles alternate with the corolla traces. The present observation, however does not support Rao (1955) who considers that the supply to the stamens and the calyx originates as a common strand. The staminal traces remain undivided upto the level of the anther and give a more or less amphicribral appearance in cross section (Figs. 31 34) In the four fertile stamens the staminal bundles trifurcate on entering the anther The resultant lateral branches move out and enter the adjoining anther lobes, while the middle one goes up and ends in the tissue of the connective. In the staminode, which is completely devoid of another the bundle ends blindly in the upper region of its tissue.

* See Lean 1953

After the traces to calyx, corolla and androecium are given out the remaining vascular bundles are arranged in manner giving an oval appearance in cross section (Fig 29) At this level fine traces are given out from these bundles to the hypogynous disc (Fig 29) After supplying the disc the central stele is seen to consist of vascular strands of unequal sizes There are two large bundles in the anterio-posterior direction of the ovary forming the two dorsal carpellary bundles (Fig 30) The other two large bundles in the plane of the septum, are the ventral carpellary bundles and are normally oriented. The strands on either side of the ventral carpellary bundles are the lateral bundles of the carpels. The lateral, as well as the dorsal and ventral carpellary bundles, give out smaller branches in the ovary wall Each of the ventral carpellary bundles gives out a branch in the region of the ovular attachment. These branches move inwards and enter the septum of the ovary (Figs. 31 32) Each one now divides into two and the resultant traces move out in opposite direction to supply the ovules. The remaining tissue of the ventral carpellary bundles divides into two (Fig 32) and the resulting branches move out laterally and disappear near the base of the style along with other lateral bundles. Thus, above the region of ovular attachment, no vascular tissue is left in the small outgrowths of the placentae. In this short region the ovary is also found to be unilocular The dorsal carpellary bundles alone pass through the style to enter the stigma (Fig 34)

Vascular supply of Nectary—The vascular supply to the nectary is given out from the branch trace It organizes to form a cylindrical stele in the stalk of the nectary (Fig 36) It may be mentioned further that the bract subtending the nectary does not receive any vascular supply (Figs. 35 36) whereas in *S indicum* (Singh, 1960) the bract receives a vascular trace The vascular cylinder of the nectary becomes dissected higher up and appears to give rise to about ten strands (Figs. 37 38) none of these enter the appendages and all are seen to terminate near the bases of the various outgrowths in the receptacle itself (Fig 35 38, 40) It has also been observed that lignified elements are not present in the distal portion of the vascular strands. Esau (1953) has mentioned that such a condition of vascular strands in nectaries is due to the presence of the secretory cells above them. Poor vascular supply to the appendages and its complete absence in the nectar bract suggest that the primordium destined to form nectary in *Pedalium murex* has undergone more modification and reduction than *S indicum* (Singh, 1960)

Nature of flower and Inflorescence—Zimmerman (1932) and Esau (1953) have shown that those nectaries which are organized structures are found to be associated with vascular tissue Nectaries in *Pedalium* have not only the vascular supplies in them but the distribution pattern of the vascular tissue is like that of flowers. The studies on organogeny of the nectaries have also revealed that the nectaries follow a course of development similar to that of flowers. Therefore, on the basis of organogeny and vascular supply of the nectaries and flowers it appears that nectaries in *Pedalium* also are morphologi-

cally floral buds with arrested growth and have undergone slight modification to suit the function of secretion like the nectaries of *S. indicum* (Singh 1960).

At the nodes in *Pedalium*, and *Sesamum* (Singh, 1960) the trace to the bract or the leaf is followed by the branch trace. From the branch traces lateral traces are given out to the nectaries in *Pedalium* and to the subtending bract and the nectaries in *Sesamum*. The remaining vascular tissue of the branch trace passes up in the flower. This continuity of the vascular system from the place of its origin upto the flower shows that the two nectaries and the flower are component organs of the same axillary branch. The terminal flower and the nectaries in *Pedalium* as well as in *Sesamum* therefore form a reduced cyme i. e., a trichome where the terminal bud has only developed into a fertile flower while the lateral buds have undergone reduction and modification for secretion.

Microsporogenesis and male gametophyte—A stamen appears as a club-shaped structure with the broader portion forming the anther and the connective while the lower portion forms the filament. The epidermis of the protuberance (Fig 41) consists of elongated glandular cells. The archesporium consists of a group of hypodermal cells at the corners of the anthers (Fig 42). Each of the archesporial cell divides periclinally to give rise to a primary parietal cell and a sporogenous cell (Fig 43). The parietal cells also divide periclinally to produce four layers forming the endothecium, two middle layers and a layer of tapetum (Figs. 44 45). The endothecium on the sides of the connective, however is more than one layered thick (Fig 50). The cells of the endothecium later develop characteristic fibrous thickenings (Figs. 48, 49 50), while the middle layers degenerate.

The tapetum is of glandular type and the layer adjacent to the connective differentiates earlier than the one of the peripheral side. The former also differs in origin from the latter as it is derived from the cells of connective side just abutting the sporogenous cells while of the peripheral side is the inner most layer of the anther wall. Later the cells of the tapetum enlarge and become multinucleate with 2-4 neclei where each nucleus has several nucleoli (Figs. 46, 47) but finally all the nuclei of a cell fuse to form a big nucleus (Fig 48). This is soon followed by the appearance of the deeply stained refractive bodies on the inner walls of the tapetal cells (Figs. 48, 49). Such refractive bodies have also been seen in *Sesamum indicum* (Singh, 1960). As these bodies increase in number the protoplasm of the cells goes on disappearing with the result that the whole layer is used up in the production of these refractive droplets (Figs. 49 50). Simultaneously with the degeneration of tapetum and the middle layers the septum dividing anther cavity also dissolves, bringing about the rupture of the anther in the plane of the septum (Figs. 51 52).

The cells of the primary sporogenous layer function directly as the microspore mother cells. The division is of simultaneous type and the result-

tant tetrads of microspores are either tetrahedral, isobilateral, decussate or T-shaped (Figs. 53 56) Each microspore first forms a bi-celled pollen grain and then by a division of the generative cell it becomes a 3-celled pollen grain (Figs. 57 58) The exine of the pollen grain shows a thickening of hexagonal pattern and has six longitudinal furrows, all of which can be seen only in the polar view (Figs 59-61) The pollen grains are oblate and hexacol pate. The behaviour of the furrows or the colpi is exactly similar to that of *Sesamum indicum* (Singh, 1960) as they get expanded when the pollen grains are exposed to moisture with the result that the cytoplasm slightly buldges out through the gaps of the furrows (Fig 61)

Megasporogenesis and female gametophyte—The ovular initials arise near the upper end of the placenta. One o two (Fig 62) archesporial cells differen tiate in the hypodermal layer near the tip of the ovular primordium, of which only one develops further At the megaspore mother cell stage the integument is initiated laterally and by the time tetrads are f rmed it surrounds the nucellus leaving only a narrow micropyle (Fig 64)

The archesporial cell functions directly as the megaspore mother cell and produces a linear tetrad of megaspores of which the chalazal one functions to form the embryo sac (Figs 65-66) The nucleus of the functioning megaspore divides to give rise first to a binucleate (Fig 67) then to four nucleate (Fig 68) and finally to eight nucleate embryosac. The latter becomes organized with an egg apparatus, two polars and three ephemeral antipodals (Figs. 69 70)

As the functioning megaspore develops to form the embryo sac the cells of the nucellar epidermis start degenerating The degeneration is followed by the differentiation of the cells of the inner epidermis of the integument into *tapetal layer But by the time embryo sac is organized the cells of the chalaza below the embryo sac also become differentiated into hypostase Srinivasan (1942) however has considered these cells merely as an extension of the integumentary tapetum.

Endosperm—The tip of the pollen tube after reaching the embryo sac be comes broad and stains deeply The presence of the remains of the pollen tube in the post fertilization stage (Fig 73) is similar to that of *S indicum* (Singh 1960)

The development of endosperm starts more or less immediately after fertilization while that of the zygote the development is slightly delayed. The first division in the development of endosperm is transverse producing a cell towards the micropyle and the other towards the chalaza (Fig 71) In the micropylar segment the first two divisions are longitudinal. This is then followed by transverse divisions giving rise to an elongated endosperm (Figs. 72, 73) The chalazal segment elongates and its nucleus divides twice to form a coenocytic haustorium (Figs. 71 73)

* Terminology used after Erdtman (1952)

After a sufficient amount of endosperm is formed the chalazal haustorium degenerates (Fig 74) This is followed by the differentiation of micropylar haustorium (Fig 75) perhaps to compensate for the degenerated chalazal haustorium In the later stages of development the micropylar haustorium also degenerates and its remains could be seen even in a mature seed No reference has been made regarding the degeneration of the chalazal haustorium and the differentiation of micropylar haustorium by Srinivasan (1942)

When the chalazal haustorium starts degenerating the endosperm becomes differentiated into three portions, namely the chalazal the central and the micropylar (Figs. 74-76) similar to that of *S indicum.* The cells of the chalazal and the microplyar portion are meristematic and appear to be responsible for the growth of the endosperm (Figs. 74 75) while the central portion consists of elongated highly vacuolated cells (Fig 76) During *further development most of the endosperm* is used up by the developing embryo sac so that only a thin layer of endosperm is left in the mature seed (Figs. 77 78) The endosperm cells contain some reserve food material.

Embryo—The zygote becomes elongated having the nucleus in its terminal portion while the basal portion contains the deeply stained cytoplasm (Fig 79) The first division of the zygote is transverse producing a terminal cell and the basal cell *cb* (Fig 80) The terminal cell *ca* next divides longitudinally and this results in the formation of inverted T-shaped proembryo of three cells (Fig 81) Transverse divisions of the cell *cb* and its derivatives give *rise to a long suspensor with its lower most cell functioning as hypophysis* initial (Figs. 82, 83) Srinivasan (1942) has made no mention of the hypophysis initial. The suspensor is gradually used up so that by the time seed becomes mature it completely disappears

The two embryonal cells derived from *ca* divide longitudinally and transversely producing eight cells arranged in two tiers (Fig 82) These divide in oblique and periclinal planes to form a globular embryo with differentiated epidermis (Figs. 83, 84) Further growth makes the embryo heart shaped (*Fig* 85) The mature embryo is spathulate and occupies nearly the whole space of the seed (Fig 86) The present observation shows clearly that the embryo develops after Onagrad type (Johansen 1950) similar to that of *Sesamum indicum* (Singh, 1960)

Seed—The integument at megaspore mother cell stage is 3-4 layered thick which becomes 10-12 layered by the time mature embryo sac is formed (Figs. 87 88) The inner epidermis forms the characteristic 'integumentary tapetum and the thickness of the integument is brought about by the div*isions of its cells*, as well as by cells of the outer layers of the integument (Figs. 88 89)

As the ovule grows older the cells of the outer epidermis become thick walled (Fig 90) In surface view these cells are polygonal in shape (Fig 91) The inner epidermis and most of the cells outer to it degenerate but the cuticle of the inner epidermis persists As the seed matures only 4 5 layers of cells of the integument are left, which along with the inner cuticle form the seed coat.

Fruit—The ovary wall in pre fertilization stages consists of 15-20 layers of cell (Fig 92) They are all of parenchymatous cells with procambial tissue distributed here and there In post fertilization stages certain characteristic anatomical and morphological changes take place in the ovary wall namely the development of glandular hairs n the epidermis, the differentiation of fibrous tissue and the development of spines.

A fully developed hair consists of a stalk and a head of four radially arranged cells (Fig 93) These hairs, however degenerate when the fruit matures The fibrous development is initiated by the divisions in the inner layers of the fruit wall. Three types of fibres arise from these cells. The fibres surrounding the ovary cavity are tangentially elongated while outer to it forming the major portion of the fibrous layer are longitudinally elongated fibres. The positions coinciding with the spines mark an abundant development of obliquely oriented fibres. The spines therefore, are formed by the strong development of fibres in association with lateral bundles of the carpels (Fig 96)

The pericarp of the mature fruit becomes differentiated into two portions The outer consists of predominantly parenchymatous cells while the inner of fibres (Figs 94 95) When the fru t dries the outer parenchymatous cells degenerate. The fibrous zone only remains which forms a thick and hard layer around the pericarpial cavity and as its constituent fibres are differently oriented, it does not show breakage at any point. Therefore, the fruit remains indehiscent.

Summary

The flower of *Pedalium murex* bear two laterally placed nectaries on its pedicel The nectaries are potential flower buds modified as organs of secretion The flower and the t o lateral nectaries form a trichome, in which the terminal bud is fertile while the lateral buds are modified into nectaries.

The floral organs develop in acropetal succession The placenta are formed at the point of contact of the semicircular carpellary initials The placentae meet in the centre and produce a bilocular ovary

The medium bundles f sepals and stamens have separate origins while the calyx laterals and the traces for the corolla arise as common traces. Vascular supply to the gynaecium consists of two dorsals, two ventrals,

and a number of lateral bundles between them The hypogynous disc receives a large number of fine traces from the central stele of the pedicel. The wall of the anther consists of epidermis a fibrous endothecium, two middle layers and a single layered multinucleate glandular tapetum. The dehiscence of the anther takes place by the dissolution of septum dividing the anther cavity The pollen grains are 3-celled, oblate and hexacolpate.

The ovules are unitegmeic, tenuinucellate, anatropous and pendulous with dorsal raphe. The embryo sac development is of Polygonum type and a mature embryo sac has an egg apparatus, two polars and ephemeral antipodal cells The inner epidermis of the integument forms the integumentary tapetum and the hypostase is differentiated below the embryo sac.

The endosperm is of cellular type with chalazal and micropylar haustoria. The chalazal haustorium is long four nucleate coenocytic structure. The micropylar haustorium is differentiated slightly later after the chalazal haustorium has degenerated Embryo develops after Onagrad type The mature embryo is spathulate in shape with two thick cotyledons and a prominent radicle surrounded by scanty endosperm.

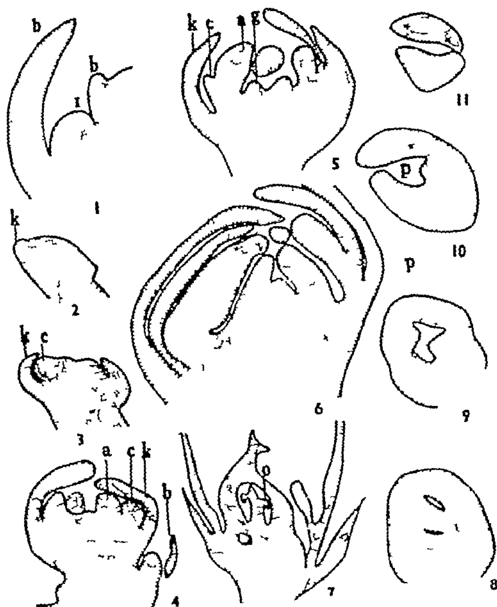
The seed coat is formed mainly by the thick-walled outer epidermis along with a few layers of degenerated parenchyma and inner cuticle. The fruit is fibrous, indehiscent and spinous. The obliquely oriented fibres in association with lateral bundles of the carpels form the spines.

ACKNOWLEDGEMENTS

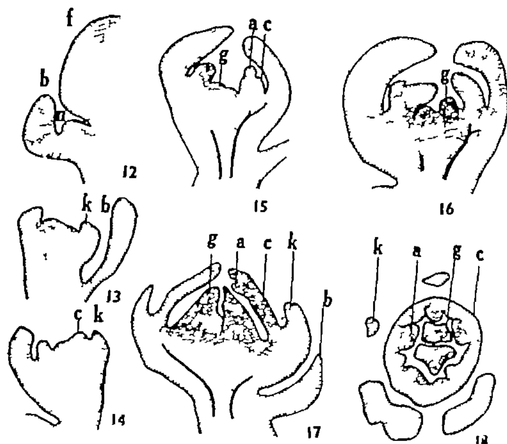
I am very thankful to Prof Bahadur Singh under whose guidance the work has been completed and to Prof P Maheshwari for the loan of some literature. In the end I express my sincere gratitude to Dr R.K. Singh, Principal, B R. College, Agra, for constant encouragement and for providing all the facilities for research

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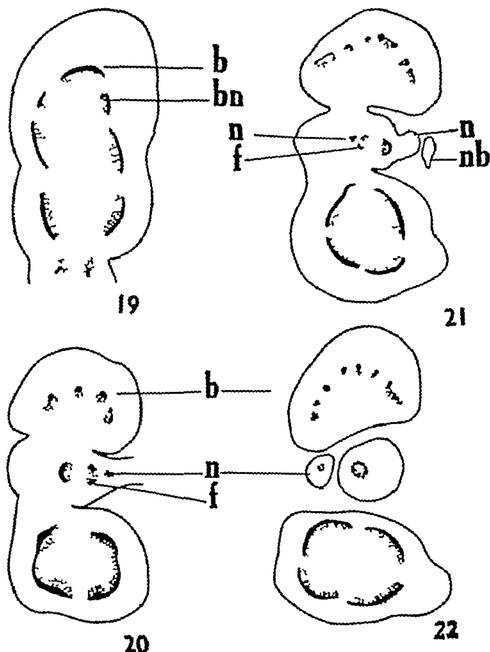
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Figs. 1-11 Organogeny of flower (a androecium b bract c corolla f flower primordium g gynaecium k calyx o ovule p placenta). Fig. 1 L.S. apex of the inflorescence showing bract and the flower primordium. Figs. 2-7 L.S. showing development of floral parts. Figs. 8-11 T.S. gynaecium initial showing the organization of placentae (For detail see text). Figs. 1-6 × 500. Fig. 7 × 100. Figs. 8-11 × 200.



Figs. 12 18. Development of nectary (*a*, appendage cf. androecium *b* bract; *c* appendage cf. corolla *f* portion of flower; *g*, appendage cf. gynoecium *k*, appendage cf. calyx, nectary primordium) Fig. 12 L. S. showing nectary initial in the axil of the bract near the basal portion of the flower Figs 13-17 L. S showing development of different whorls of appendages. Note the similarity of Fig 17 with that of the flower bud (Fig 6) Fig. 18. T S. nectary showing the four whorls of ppendages Note the pentamerous nature of outer three whorls cf. calyx corolla and androecium and the inner of only two ppendages cf. carpels. Figs. 12 17 X 500. Fig 18. X 200.



Figs. 19-22. (*b* bract; *bt*, bract trace; *bn*, branch trace; *f* flower supply; nectary supply *nb* nectary bract) T. S. node showing the origin of vascular supply to flower and nectary. Note the absence of vascular tissue in the bract subtending the nectary. All × 80. (For explanation see text.)

Figs. 23-34. Vascular supply of flower (staminal supply *br*, branches from the ventral carpellary bundles; corolla trace; *d*, dorsal carpellary bundles; *f* fibrous tissue; *ll*, calyx laterals; *lm*, calyx median; position of the posterior staminode; supply to the hypogynous disc; *v*, ventral carpellary bundle).

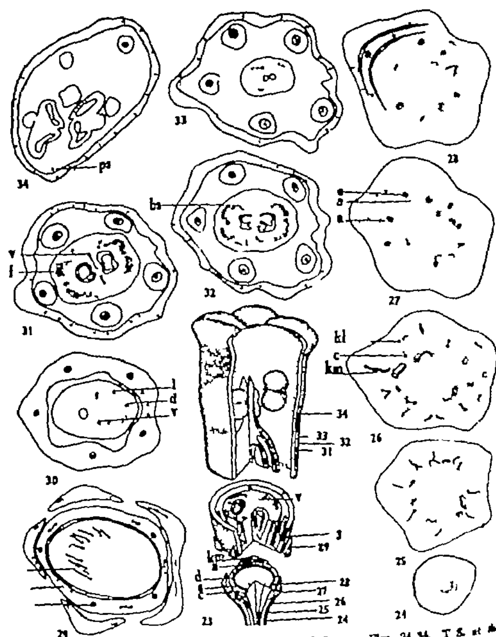
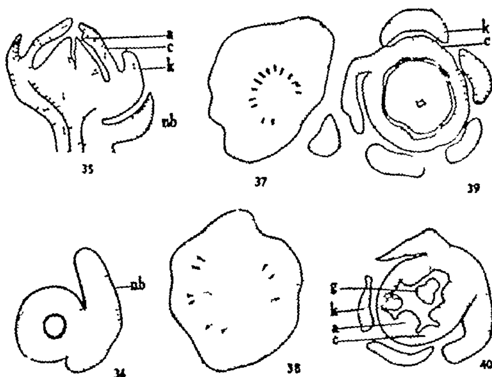
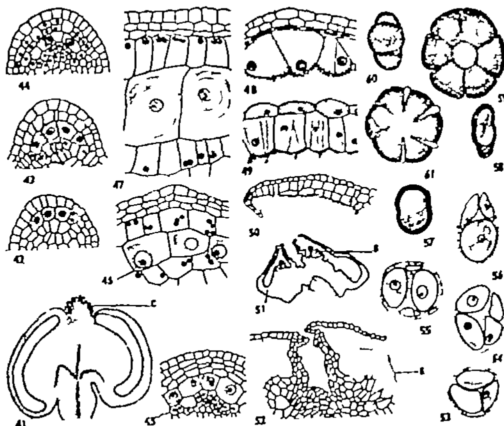


Fig 23. Diagrammatic perspective view of flower Figs. 24-34. T. S. at the levels as marked in Fig 23. Figs. 24-34 X 100 (For explanation see text)



Figs. 35-40. Vascular supply of ectary (appendage cf. stamen; ppendage cf. corolla; g appendage cf. gynaecium; k ppendage cf calyx at bract of nectary) Fig. 35 L. S nectary showing the pproximate levels from which T S. have been drawn. All × 200 (F explanation see text)



Figs. 41-61 Megasporogenesis and development of male gametophyte (c glandular connective degenerated septum of the anther) Fig. 41 L.S. stamen showing glandular connective anther and filament X 100. Figs. 42-50. T S. showing development of sporogenous tissue and wall layers of anther. Fig 42-49 X 300 Fig. 50 X 100 Figs. 51 52. Dehiscence of anther wall. Fig. 51 X 20 Fig 52 X 100 Figs 53-56. Tetrads X 300 Fig 57 Bicelled pollen grain X 300. Fig 58 Tricelled pollen grain X 300 Figs. 58-60 Polar and equitorial view respectively of the pollen grains. X 300 Fig 61 Pollen with wide pen Colpi X 300

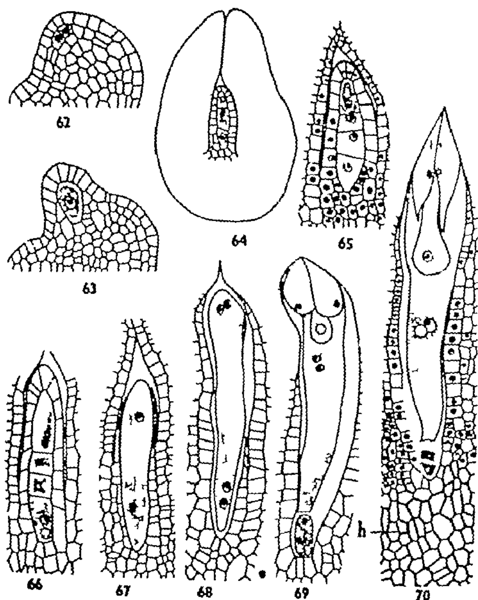
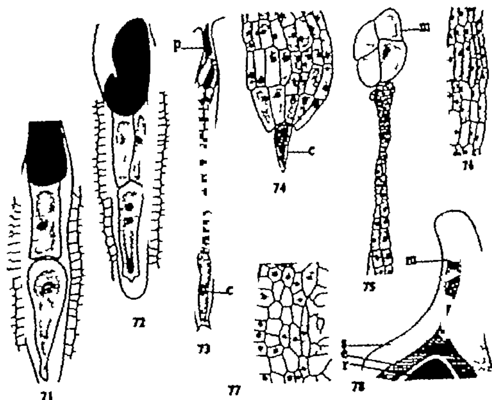
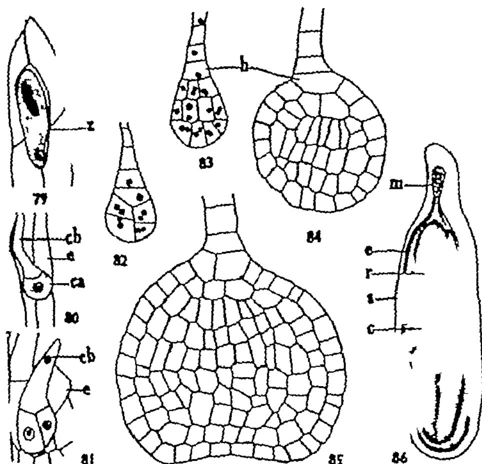


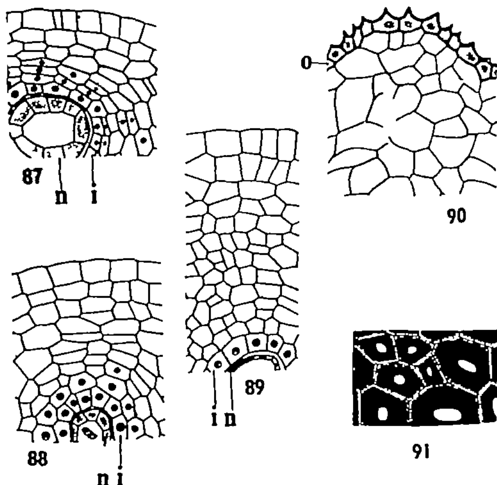
Fig. 62-70. Megasporogenesis and female gametophyte (*A. hypostase*) All X 1500 except Fig. 64 X 1000



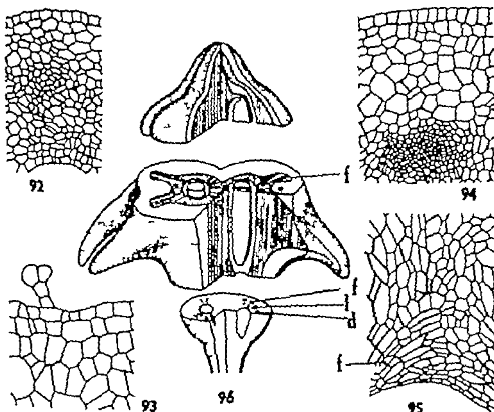
Figs. 71-78 Development of endosperm (chalazal haustorium; endosperm m, micropylar haustorium p persistent pollen tube r radicle s, integument Figs. 71-72 initial stages in endosperm development. X 1500 Fig 73. Endosperm with chalazal haustorium X 800 Fig 74, degenerated chalazal haustorium and a portion of the endosperm X 1000. Fig 75 Micropylar haustorium and a portion of endosperm X 500 Fig. 76. Central portion of the endosperm. X .00 Fig 77 Endosperm from a nearly mature seed X 1000 Fig 78. L. S micropylar part of seed showing degenerated micropylar haustorium abutting the endosperm. X 100.



Figs 79-86. Development of embryo (cotyledons; endosperm ; m, degenerated micropylar haustorium; radicle; z, seed coat). Figs 79-83 X 1500. Fig 86. X 50



Figs 87-91 Development of seed coat (*i* inner epidermis *n*, nucellar epidermis; *o*, outer epidermis) Figs. 87-89 T. S. integument of pre-fertilization stages X 1500. Fig 90 integument in post-fertilization stage X 500 Fig. 91, epidermal cells of seed coat in surface view X 650



Figs 92-96. Development of fruit (*d*, dorsal carpellary bundle; *f* fibrous cells; *l* lateral bundles) Fig 92 T S. ovary wall in pre-fertilization stage. Fig. 93 T S ovary wall in post-fertilization stage showing an epidermal hair Figs 94 95. T S showing outer and inner respectively of fruit wall All × 1000 Fig 96 Diagrammatic perspective view of mature fruit showing the association of fibres and the carpellary bundles in formation of spines. Note the positions of strong fibrous development and the lateral bundles of the carpels

GONIOMETRIC STUDY OF DYSPROSIUM SULPHATE OCTAHYDRATE

RUPENDRA KUMAR KASHYAP
Physical Laboratories Agra College Agra

ABSTRACT

With a two circle goniometer the interfacial angles of $Dy_2(So_4)_3 8H_2O$ crystals were measured. Modifying the illuminating system of the goniometer faithful measurements could be obtained even from striated faces From the observed interfacial angles we obtain a b c = 3 0159 1 :2.0160 and $\beta = 118°28'$ agreeing well with the values of the other members of the series

INTRODUCTION

In recent years the physical properties of the isomorphus series of the octahydrated sulphates the halides, the ethyl sulphates of rare earths ions have focussed the attention of a large number of workers. A large amount of data on magnetic susceptibility paramagnetic resonance absorption, optical absorption etc. have been collected Crystallographic data by X-ray methods are available for crystals like anhydrous $DyCl_3$ (Zachariasen 1948) $Sm_2(So_4)_3 8H_2O$ (Zachariasen 1935) Ethyl sulphates (Keteler 1937) and double nitrates (Duffus and Wolf 1953) Morphological crystallographic data are available only for the octahydrated sulphates of Pr Nd Gd, Sm, and Er (Groth 1908) and $Smcl_3 6H_2O$ (Pabst 1931)

The crystallographic data of goniometric studies for the octahydrated crystals which are useful for X ray and magnetic studies are not available for other members of the series. Hence the present author has undertaken the goniometric studies of the octahydrated sulphates of Eu, Dy Ho Tb and Yb. This will be useful for the magnetic susceptibility measurements which are in progress in this laboratory

EXPERIMENTAL

The crystals of $Dy_2(So_4)_3 8H_2O$ were obtained by slow evaporation from slightly acidic aquous solution in a dust free chamber Chemical used was of E. Merck G R. variety and triple distilled water was taken for preparing the solution After several days a crop of good crystals came out. These were redissolved and recrystallised, when very good crystals were obtained. From this crop a few well defined crystals weighing about 0 005 gms were picked up and tested under a polarising microscope for any probable twinning Finally ten good single crystals were selected for goniometric study

The two circle goniometer used for the study of $Dy_2(SO_4)_3 8H_2O$ was modified to give better and clear reflections even from striated surfaces of the crystals and thus avoided the necessity and labour of selecting the crystals very accurately from the large number for goniometric purpose as was in usual practice. And this was achieved by introducing a new form of cross-wire 'Crossed Filament Bulb (Fig 1) in place of simple non-luminous cross-wire in collimator The image of this crossed filament which was produced in the telescope after the reflection from the crystal surface was bright on a dark background (while the image of non-luminous cross-wire was dark) and so could be seen and located much more easily and clearly

RESULTS

(a) Crystal —

Monoclinic, prismatic, 2/m. a b c=3.0158 1 2 0160 β=118°28'

(b) Forms Represented —

Orthopinacoid {100} and Basal pinacoid {001} {10$\bar{1}$} {11$\bar{1}$} and {31$\bar{1}$}

(c) Interfacial Angles —

The normal crystallographic angles taking symmetry considerations and good reflections are as follows. The notations for faces are according to Groth.

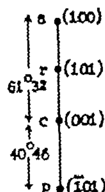
Angles	Measured value of angles	Calculated value of angles
ρ:a=(10$\bar{1}$) (100)	77°42	——
a:c=(100) (001)	61°32	——
c ρ=(001) ($\bar{1}$01)	40°46	——
ρ:ω=(10$\bar{1}$) (11$\bar{1}$)	63°5	——
a:r=(100) 101)	37°42	37 31
a:ω=(100) (11$\bar{1}$)	84°20'	84°28
a.ζ=(100) (31$\bar{1}$)	52°26	——
a.q=(100) (011)	——	76°27
c.q=(001) (011)	——	60°84

DISCUSSIONS

(a) *Calculations of Interfacial Angles and Indexing the Faces* —

The stereogram is shown in Fig 3 The crystal is isomorphous to other rare earth sulphates which have been studied by Groth The faces a(100) and c(001) have been chosen according to $(SO_4)_3Er_2 8H_2O$ crystal (Groth

1908) and have been varified magnetically also. The face ρ' is $(10\bar{1})$ and so ω will be $(11\bar{1})$

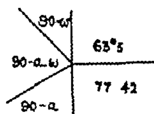


The angle a (100) r (101) can be calculated as follows

$$\frac{\sin a \quad r}{\sin 61\ 32'} \times \frac{\sin 40° 46'}{\sin \rho \quad r} = \frac{\begin{matrix}100100\\101101\end{matrix}}{\begin{matrix}100100\\001001\end{matrix}} \times \frac{\begin{matrix}\bar{1}01101\\001001\end{matrix}}{\begin{matrix}\bar{1}01\bar{1}01\\101101\end{matrix}} = \frac{1}{2} = \frac{p}{q}$$

p. cot a r − q cot a c = (p − q) cot a ρ

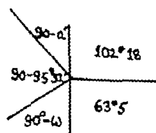
from which a(100) r (101) = 37°31



The angle a(100) ω $(1\bar{1}\bar{1})$ is obtained from Naplerian triangle Fig (4)

cos a ω = cos 63°5 cos 77°42'

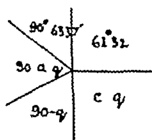
Therefore a ω = a(100) : $\omega(11\bar{1})$ = 84°28



Now from Naplerian triangle aω ρ Fig (5)

cos a = tan (−5°32') tan (90+12°18')

therefore a = 63°37



Again from Naplerian triangle aqc' Fig (5)

cos 63 37 = tan 61°32 cot a q

therefore a(100) q(011) = 76°37

and sin c q = sin 76°27 sin 63°37'

therefore c (001) q (011) = 60°34

Now from zone ω q a ξ

↑ ω′ ($\bar{1}11$)
19°5′
q (011)
76°27′
a (100)
52°26′
ξ (hkl)

$$\frac{\sin 19°5}{\sin 95\ 32} \times \frac{\sin 52°26'}{\sin 128°53} = \frac{\begin{matrix}\bar{1}11\bar{1}11 \\ 011011\end{matrix}}{\begin{matrix}111111 \\ 100100\end{matrix}} + \frac{\begin{matrix}hklhkl \\ 100100\end{matrix}}{\begin{matrix}\overline{hklhkl} \\ 011011\end{matrix}}$$

or $0.3346 = 1/h = \bar{k}/h$

the ratio being a commensurable number

therefore $1/3 = 1/\bar{h} = \bar{k}/h$ or $h/3 = \bar{l}/1 = \bar{k}/1$

therefore $hkl = 3\bar{1}\bar{1}$

(*b*) *Axial Ratios* —

$$\frac{c}{b} = \frac{\tan (001)\quad (011)}{\sin \beta} = \frac{\tan 60°34}{\sin 118°28} = 2.0160$$

$$\frac{c}{a} = \frac{\sin (001)\ (101)}{\sin (100)\ (101)} = \frac{\sin 24\ 1}{\sin 37°31} = 0.66843$$

$$\frac{a}{b} = \frac{a}{c} \times \frac{c}{b} = 3.0158$$

therefore a b c = 3 0158 1 2.0160

and β = 118°28

It is thus seen that these values of a b c and β agree well with other octahydrated isomorphous crystals Pr Nd, Er etc. (Groth 1906)

ACKNOWLEDGEMENTS

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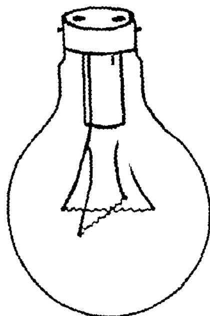


Fig. 1 Crossed Filament Bulb

Fig. 2

Fig 3

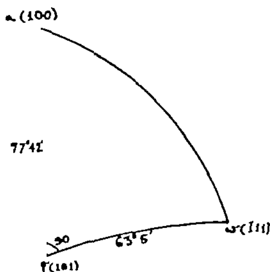


Fig 4

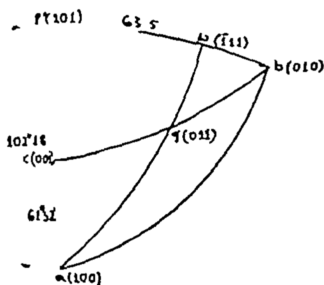


Fig. 5

HIGH ALTITUDE INSECTS

M S MANI

INTRODUCTION

High altitude insects are an ecologically highly specialized, mountain autochthone petrophile, cold stenotherm animals that inhabit the elevated regions above timber-line on mountains The timber line is the altitudinal zone where the forest gives place to open vegetation On mountains situated in the torrid zone, especially close to the equator the timber-line lies usually between 3050 and 3700 metres above mean sea level, but in the temperate latitudes the upper limits of the forest are reached at elevations between 1500 and 2000 metres above mean sea level. On the Himalaya the timber line is between 3000 and 3700 metres, on the Alps at about 2000 metres on Andes at about 3690 metres, on Mt. Rainier at an elevation of 1970 metres and between 400 and 900 metres on North Scandinavian mountains. Although thus the timber-line varies within wide limits, the insects habitually existing in the treeless zone at elevations above 2500-3000 metres above mean sea level should properly be considered as typically high altitude forms.

The high altitude environment is characterized by reduced atmospheric pressure, low atmospheric temperature often below freezing point, high atmospheric aridity and rapid rate of desiccation from exposed surfaces, intense insolation and radiation, relatively high ultra-violet intensity high wind velocity more or less prolonged snow cover scanty soil, ruggedness of terrain and short vegetative season As recently discussed by Troll[23] and Büdel[17] the general high altitude ecological conditions differ considerably depending upon the situation of the mountain in the torrid zone or in the temperate latitudes. The greatest bulk of the typical high altitude insects are generally small-sized, flightless or apterous mostly heavily pigmented terricole forms that occur under stones, in the immediate vicinity of glaciers snow and melt waters. There is on the whole a general preponderance of predatory necrophagous or other carnivorous types.

The high altitude insects exhibit a remarkable and characteristic altitudinal stratification of species and their abundance also diminishes with increase of altitude until relatively few occur near the snow line. The snow-line, like the timber-line varies from 5500 metres on Mt. Kilimanjaro to about 2590 metres on the Alps and sinks to sea level in arctic regions. On the Himalaya the snow-line lies at about 5200 metres and on the Karakoram at about 5600 metres. The snow-line does not however represent the upper altitudinal limit of insect life on mountains. Diverse insects flourish very

much above the snow-line both on the Alps and on the Himalaya. As at present known, the highest elevation at which insects habitually exist in the world is somewhat above 6000 metres on the North West Himalaya[9]. The horizontal distribution of high altitude insects is characterized by pronounced discontinuity localized concentrations isolation and high endemism often on single mountain peaks many of which have also characteristic local races or varieties. The dominant high altitude insects are Collembola Coleoptera especially Carabidae, Staphylinidae Tenebrionidae and Curculionidae Diptera Lepidoptera like *Parnassius* Latr., *Argynnis* Fabr and *Colias* Fabr., etc. Some unique Dermaptera and Acrididae are also often met with at very high elevations. The melt water torrents abound in Ephemerida Plecoptera, Trichoptera and aquatic Diptera. Nearly every insect community contains also numerous mites, spiders scorpions, chelifers, Symphyla and centipedes

Although high mountains exist in nearly every part of the world only the Alps may be said to have been more or less fully explored by entomologists Among the other more well known mountains from which some high altitude insects are known are the Himalayan System, the Alai Pamir the Caucasus, the Carpathian the Pyrenees, the Apennine, the Atlas Mountains, the Rocky Mountains the Alleghenian Range the Andes and the East African mountains like Mt. Kilimanjaro Mt. Elgon Mt. Kenya, Mt. Meru and the Ruwenzori Range. Some of the Abyssinian mountains have also recently been entomologically explored. Some of the outstanding features of insect life at high altitudes, with special reference to the Himalaya, have been recently summarized by Mani[14] Study of high altitude insects may be said to have been sadly neglected so far The number of species of typical high altitude insects so far known from the world does not in any case exceed 5000 Of these, nearly 1500 species are recorded from the Alps and other high mountains of Europe about 1500 from the Himalayan System, 200 from African mountains, 300 from the Rockies and other North American mountains, 300 from the Andes and about 200 from various other mountains. Recent experience in the Himalaya has shown that unexpectedly larger numbers of species than known at present do actually exist at extreme high elevations and this is probably true also of nearly every other high mountain in the world.

High Altitude Insects from the Torrid Zone

The principal mountains of the torrid zone so far explored entomologically include the East African mountains viz Mt. Kenya (altitude 5194 metres, 0° 28 S L.) Mt. Kilimanjaro (altitude 5965 metres 3° 55 S. L.) Mt. Elgon (altitude 4321 metres 1 N L.) the Ruwenzori Range (altitude 5100 metres, 0° 25 N L.) the Ethiopian mountains Mt. Kinabalu in North Borneo (4097 metres above mean sea level and at 6° N L.) Mts. Mauna Kea (altitude 4250 metres 19° 35 N L.) and Mauna Loa (altitude 4168

metres, 19° 30 N L.) in Hawoii Mt Pinchincha (4775 metres) almost on the equator Mt. Antisana (5660 metres 1 8 S L.) and Mt. Cotopaxi (5880 metres, 1° S L.) and Mt. Chimborazo (6255 metres 1 30 S L.) on the Andes in Ecuador (South America) Mt Huascaran (6762 metres 9° S. L.) on the Andes in Peru and Mt Orizaba (5546-5698 metres 19° N L.) in Mexico. A part of the Rocky Mountains in North America is also within the torrid zone. The Himalaya, situated between 27 and 35 N L. almost near the fringe of the torrid zone is however considered under the Himalayan System of the temperate zone.

The East African Mountains.—Although numerous scattered references to insects from the mountains of equatorial East Africa are found in literature, the most important contributions to our knowledge of the high altitude insects of Mt. Kilimanjaro, Mt Kenya Mt. Meru and the Ruwenzori Range are by Alluaud[2,3] Alluaud and Jeannel[4] Butler[19] Chanel[21] Fairmaire[24] Godman[30] Jeannel[43,44] Johnston[49] Kolbe[51] Meyer[69] Salt[78] Sjöstedt[76,77] Waterhouse[83] and the British Museum Ruwenzori Expedition[14] On Mt. Kilimanjaro the forest extends up to an elevation of about 2600 metres on the eastern slope and 3000 metres on the westren slope. Both near the upper limit of the forest and sometimes even above this limit there are isolated patches of *Erica arborea* Linn so that the timber line on Mt. Kilimanjaro is not strictly comparable with that on the European mountains. The alpine-prairie zone extends on Kilimanjaro from the timber line to nearly an elevation of 4200 metres above mean sea level, above which lies the alpine-steppe zone. The principal peak on Kilimanjaro is the snow-covered Kibo Mawenzi is a lesser peak and there is a third peak Kifimiko, which is only 3500 metres above mean sea level. The glacier zone is above an elevation of 5000 metres

It is of considerable importance to observe that the insect life above the timber line on Mt. Kilimanjaro is richer than on Mt. Kenya or even on the Ruwenzori Range. This wealth of insect life on Kilimanjaro seems to be related to the fact that on this mountain the soil above the timber line is not swampy and is thus optimal to much wider terricole forms than on Mt. Kenya or on the Ruwenzori Range on both of which local swampy conditions seem to be rather unfavourable for the development of an extensive terricole fauna

Although widely distributed mountain genera like *Bembidion* Latr *Agonum* Bon. *Atheta* Thoms., etc., are found on Kilimanjaro, it must be noted that there is no affinity between the high altitude Coleoptera of Mt. Kilimanjaro and the European mountains. The high altitude Carabidae on Mt Kilimanjaro are particularly remarkable for the minute apterous subgenus *Orinodromus* Kolbe of *Calosoma* Weber related to *Carabophanus* Kolbe from the Abyssinian highlands *Calosoma* (*Orinodromus*) *deckeni* Gerst. occurs on alpine meadows, at elevations of about 2600-3000 metres on the south-east slope of Mt. Kilimanjaro. Alluaud (*loc cit*) reports that *Calosoma* (*Orino-

dromus) *glacialis* Kolbe occurs at an elevation of 4000 metres above mean sea level. The species of *Orthodromus* Kolbe are non-metallic, generally dark reddish-brown or also black. Among the other high altitude Carabidae known from Mt. Kilimanjaro mention should be made of the following *Bembidion kilimanum* All (2600-3000 m) *Tachys ascendens* All. (2600-3000m) *Plocamotrechus kilimanus* Jeannel (2800 m) *Zagrochilus bedeli* All. (2800 m) *Ornnophonus kilimanus* All (2800 m) *Cymindis kilimana* Kolbe (2600-3000 m) *Hystrichopus alticola* All (2600 2800 m) *Metabletus kilimanus* All (2800-3000 m) and *Zuphium ascendens* All. (2800 m) The Dytiscids *Hydroporus* (*Nebrioporus*) *kilimandjarensis* Reg and *Agabus dytiscoides* Reg have also been reported at elevations between 3000 and 3500 metres The principal Staphylinidae include *Homalium algidum* Fauv., *Staphylinus dispersus* Fauv., *Philonthus alticagans* Fauv *Atheta praticola* Fauv and *Tachyusa pratensis* Fauv The Tenebrionid *Phrynocolus ater* Wat., occurring at elevations between 3000 and 4000 metres, is so far known only from Mt. Kilimanjaro A number of Curculionidae like *Parasystates minor* Auriv *Hyposmias lobeliae* Auriv *Oreorrhytus* spp and *Cossonus lobeliae* Auriv are also recorded from above the timber line on Mt. Kilimanjaro The high altitude Lepidoptera of Kilimanjaro are conspicuously poor in typical mountain species. *Cupido aequatorialis* Sharp found on Kilimanjaro up to 4000 metres occurs also on other equatorial African mountains like Mt Elgon at elevations of 2590 metres on Ruwenzori, Mt. Kenya and Mt Birunga and is perhaps one of the few typical mountain forms The other species like *Vanessa cardui* Linn., usually found above timber line on Kilimanjaro occur also commonly at much lower elevations and even on the plains No Satyridae seem to have also been found so far but Sjöstedt collected several moths like *Phryganopsis elongata* Auriv (Lithosiidae) *Barsilia eriopygoides* Auriv (Noctuidae) *Oreometra vittata* Auriv *Hypsometra ericinellae* Auriv *Hydrelia costalis* Auriv *Triphosa corticearia* Auriv *Onychia* (*Eubolia*) *vicinaria* H. Sch *Larentia sjostedti* Auriv (Geometridae) *Gorgopsis alticola* Auriv (Hepialidae) etc., some of which seem to be particularly confined to the elevations above timber-line. The apterous Dermapatera *Forficula sjostedti* Burr found at elevations between 3000 and 4000 metres, is common under stones above the timber-line, but has also been collected within the rain-forest zone at an elevation of 2000-3000 metres on Kilimanjaro It also occurs at elevations of 4000-4300 metres on Mt. Meru A few Acrididae like *Heteropternis coelestina* Saus., *Parasphena pulchripes* Gerst. *Chrysochraon kilimandjaricus* Sjöstdt. are known exclusively from above the timber-line, at elevations of about 3000 and 3500 metres on Mt. Kilimanjaro Collembola occur at very high elevations and Sjöstedt found *Mesira annulicornis* Börner at about 3800 metres. According to him they are most abundant on lichen incrusted stones at the edge of permanent snow at an elevation of 5500 metres Salt (*loc cit.*) has also recorded species of *Hypogastrura* Bourl at high elevations on Kilimanjaro

Our knowledge of the high altitude insects of Mt. Kenya is based largely on the work of Alluaud and Jeannel[4] They have recorded a number of interesting subterranean Coleoptera from the edge of the forest. The most interesting of these finds are perhaps the blind Carabid *Scotodipnus jeanneli* Alluaud occurring at elevations between 2600 and 2800 metres, the Pselaphid *Jeannelius microphthalmus* Raffr., remarkable for its very minute and unpigmented eyes and blind Staphylinidae and microphthalmic Curculionidae. Among the Coleoptera occurring far above the timber line on Mt. Kenya the most remarkable are unquestionably *Bembidion mackinderi* Alluaud (3800-4400 metres) *Plocamotrechus kenyensis* Jeann (4000-4500 metres) *Kenyacus acrobius* Alluaud (3500-3750 metres) and *Kenyacus hypsibius* Alluaud (2800-3500 metres) It is of considerable interest to observe that the genus *Plocamotrechus* Jeann. is known also from South Africa and, as mentioned above, is also represented by a single species on Mt. Kilimanjaro Mt. Elgon and the Ruwenzori Range and thus appears to be a typical mountain autochthone form that has spread to the temperate south. Jeannel records a second species *Plocamotrechus elgonicus* Alluaud on Mt. Elgon. *Trechus gestedti* Alluaud has also been found at higher elevations on Mt. Meru. It is remarkable that while apterous *Calosoma* Weber is absent on Mt Kenya, the apterous *Calosoma* (*Carabomorphus*) *calcaratum* Roeschke extends on the Aberdare Mountain from the edge of the forest to the alpine meadows at elevations of 3000-3100 metres.

A fact of considerable ecological interest is that compared to the high altitude insects of the central and south European mountains, those on the tropical high mountains of Africa are relatively dull coloured

The Ethiopian mountains.—The Ethiopian mountains differ conspicuously from the East African mountains in a number of features. The mean elevation of the Ethiopian highlands is great, but the altitudes of isolated peaks are less than those of the East African mountains like Mt. Elgon, Mt. Kilimanjaro Mt. Kenya and the Ruwenzori Range. The highest massif in the Ethiopian highlands is the Degien Masiif which rises to an elevation above 4575 metres above mean sea level. *Mt. Bushit rises to an elevation of 4267 metres*, Mt. Batu exceeds 4267 metres and a number of other mountains are only about 3657 metres to 3960 metres high. Mt. Abuna Yosef was entomologically explored by Raffray[44] A number of insects were recently collected by Marches Saverio Patrizi in 1940 from an elevation of 3250 metres on Mt. Termaber situated about 70 kilometres from Addis Ababa on the road to Dessie. We are indebted to Raffray[4] Basilewsky[11, 12] Dyte[23] Jeannel[44] Marshall[54] Scott[73, 4] and Uvarov[83] for our knowledge of the high altitude insects from the Abyssinian mountains. Some of the genera occurring at elevations between 3500 and 4000 metres above mean sea level on the Ethiopian mountains are also known from the alpine zones of the principal central and south European mountains. On the Lasta Mountain

Range in Abyssinia, at an elevation of about 3500 metres, Raffray (*loc.cit.*) recorded many species of *Calosoma* Weber like *Calosoma carabeides* Raffr which have distinct *Carabus*-facies A species of *Cymindis* Latr is closely related to *Cymindis humeralis* two species of *Harpalus* Latr are related to the European *Harpalus gendeli* and *Harpalus litigiosus* the species *Amara analoga* to *Amara trivialis* from Europe three species of *Calathus* Bon., one of which seems to be related to the Californian *Calathus ruficollis* and also two species each of *Trechus* Clairv and *Bembidion* Latr Two species of Dytiscidae related to *Agabus cephalotes* from Corsica are found under submerged stones in icy-cold torrents The apterous subgenus *Carabophanus* Kolbe of *Calosoma* Weber found above timber line on the Abyssinian mountains, is not however represented in Europe. Among the Staphylinidae the species of *Ocypus* Steph. from the Abyssinian mountains are related to the European *Ocypus fulvipennis* A species of *Deleaster* Erichs occurring both in Europe and in North America, has been found on the Ethiopian highlands Two Abyssinian high altitude species of Curculionidae, viz *Otiorrhynchus* Germ. are related to *Otiorrhynchus pardix* Oliv from the Tirolian Alps and one to *Otiorrhynchus* ... from the Pyrenees. Scarabaeid genera, totally unknown in Europe, but found above the timber line on the Abyssinian mountains, include *Semilogonus Schizonycha*, *Centrontyx* Fairm, etc. Paussidae are particularly abundant at higher elevations on the Abyssinian highlands more than in the sub-alpine zone where they are wholly absent. Many of the Paussidae of the high altitudes of Abyssinia are strictly endemics Except *Trechus* Clairv *Calathus* Bon and *Amara* Bon., the affinities of most other Carabid genera are entirely African. The boreal forms which have extended their range to the Ethiopian highlands, become poorer and poorer as we proceed to the south. There are, for example, over 17 endemic species of *Calathus* Bon. on the Ethiopian mountains, but none further south. Fifteen endemic species of *Trechus* Clairv (*s str*) have been found on the Ethiopian highlands and a number of species have also evolved on Mt. Elgon and still further south we have *Trechus sjöstedti* Alluaud on Mt. Meru *Trechus gughensis* Jeannel is the southernmost species from Mt. Elgon so far found in Ethiopia The ancestral stock of *Trechus* Clairv in Africa seems to have developed primarily in the moist forests at lower elevations and the existing montane species began as colonizations from such types. The ancestral stock of the African *Trechus* Clairv extended also southwards. The tropical African species of *Plocamotrechus* Jeannel radiated northwards from a southern dispersal centre during the Miocene and the species of this genus are at present abundant at all elevations in South Africa. While many relict species occur on Mt. Kilimanjaro Mt. Aberdare Mt. Kenya and particularly on the Ruwenzori Range, none is however known at present on the Ethiopian highlands. Both *Trechus* Clairv and *Plocamotrechus* Jeannel are absent in tropical Africa and are confined to only the mountain ranges of the western and eastern branches of the Rift Valley system. *Omotaphus* ... Basilew found between 3050 and 4267 metres, is related to forms from the Aberdare Mountain and Mt. Elgon and *Lasgonum scotti* Basilew Is related to species

from Mt. Kilimanjaro. All the species of *Trechus* Clairv from the Abyssinian highlands, including *Trechus sublaevis* Jeannel and *Trechus bipartitus* Jeannel from Mt. Abuna Yosef described by Raffray and four species from Mt. Chillalo and *Trechus gughensis* Jeannel from the Gughé Highlands are apterous. Even *Trechus aethiopicus* Jeannel known from relatively lower elevations like 2800 metres, is apterous. Many species of *Calathus* Bon. are likewise apterous. Some of the species are indeed known only from particular massifs The four species of *Trechus* Clairv recorded from Mt. Chillalo do not, for example occur elsewhere, but other species occurring at lower elevations are rather more widely distributed. According to Hugh Scott[73 74] more than half the high altitude Coleoptera from the Ethiopian Highlands have either greatly atrophied wings or they are fully apterous.

Although several species of Lepidoptera have been collected at about 3350 metres the highest elevation at which they have been found on the Ethiopian highlands is 4267 metres. Some boreal forms like *Colias electo* Linn. *P. atia deplidice aethiops* J & V., *Lycaena phaeas pseudophlaeas* Lucas *Pararge maderakal* Guerin generally occur at elevations of about 3350 metres *Vanessa cardui* Linn occurs at about the same elevation and *Argynnis hyperbius* R. & J occurs on the southern Ethiopian mountains.

Extremely few species of ants seem to have been found at elevations above 2400-2750 metres in Ethiopia. Only exceptionally rarely *Engramma ilgi* Forel and *Acantholepis capensis* Mayr are found at elevations of 3200 metres on the Deglen Massif. The high altitude Diptera from the Ethiopian highlands include the wingless Sphaerocerid fly *Bisorbitalia tristis* Richards[70] related to two other species known from the Gughé Highlands (6° N.L.) and the Aberdare Mountains (Kenya) separated by a gap of nearly 720 kilometres, at elevations ranging from 3650 metres and more

The Andes —Among the high altitude insects from the part of the Andes, situated within the torrid zone, especially on Mt. Chimborazo, Coleoptera have been rather more fully studied than any other order[86] Troll[83] has recently given a brief account of the salient ecological conditions and zonation on the Andes in the tropical region. The snow-line on Mt. Chimborazo is at an elevation of about 4880 metres. The typical high altitude Coleoptera of Mt. Chimborazo are species of *Pterostichus* Bon. *Trechus* Clairv., *Bembidion* Latr., *Silis* Latr and *Erirrhinus* Schönh., some of which genera have several endemic species on high mountains in the Palaearctic also *Paltnaldius* Bates is a Neotropical genus from the Andes, with one transgressive species in Arizona and New Mexico. It is remarkable that no *Calosoma* Weber and Chrysomelidae have so far been found at high altitudes on Mt. Chimborazo. The genus *Colpodes* M L. is widely distributed in the warmer parts, but absent in Europe and most parts of the Nearctic The genus, found up to the snow-line on Mt. Chimborazo seems to have devloped similar ecological

features like *Nebria* Latr or *Platynus* Bon According to Whymper[41] *Colpodes megacephala* and *Colpodes pinchinchae* were found among stones cemented together by ice on the highest peak Pinchincha (4775 metres) situated almost on the equator in Ecuador Most of the high altitude Coleoptera from the region are small-sized and generally also dull coloured The following are some of the more interesting records from Mt. Chimborazo *Pelmatellus andium* Bates (3352-4115 metres) *Pterostichus (Agraphoderus) calicensis* Bates (3600-4050 metres) *Pterostichus (Agraphoderus) liodes* Bates (2740-4050 metres), *Pterostichus (Agraphoderus) integer* Bates on the south and east slopes (3530-4050 metres) *Colpodes megacephalus* Bates on the summit ridge of Guaga Pinchincha (4755 metres) and other localities at elevations from 3657 to 4267 metres *Colpodes pustulosus* Bates *Colpodes fuscipalpis* Bates and *Colpodes rotundiceps* Bates on Cayambe (4372 metres) *Colpodes pinchinchae* Bates on Pinchincha (4572 metres) *Colpodes orcus* Bates on the western slope (4575 metres) *Colpodes diops* Bates on Pinchincha (4267-4575 metres) *Colpodes steno* Bates at Cayambe 4573, Pinchincha (4267-4725 metres) *Colpodes alticola* Bates (2745-4050 metres) *Trechus* sp at Cayambe (4575 metres) *Bembidion fulvocinctum* Bates an apterous species (3657-4575 metres) *Bembidion andinum* Bates on the western slope (4820 metres) *Uroxys elongatus* Har (Coprinini) on Cotopaxi (3657-4115 metres) and from Quito *Clavipalpus antisanae* Bates from Hacienda of Antisana (4050 metres) (Melolonthidae) *Platycoelia nigricauda* Bates from Cotopaxi (3657 metres) Hacienda of Antisana (4050 metres) (Rutelidae) and the Curculionids *Helicorrhynchus vulsus* Olliff. from Pinchincha (4757 metres) and Chimborazo (4817 4876 metres) an endemic genus related to the Holarctic *Otiorhynchus* Germ. *Amphacitus porticollis* Olliff from Cayambe (45/5 metres) and Chimborazo (4817 metres) *Listroderes uncinipennis* Olliff from Cayambe (4575 metres) *Amathynetes alticola* Olliff Chimborazo (3657-4817 metres) and *A. nivalis* Olliff. Chimborazo (4575 metres) (genus *Amathynetes* endemic to the region and related to *Listroderes* Schönh); *Macrops cedorum* Olliff. Pinchincha (4720) and Chimborazo (4817 metres); *Hilipus longicollis* Olliff Hacienda of Antisana (4050 metres) *Erirrhinus glaber* Olliff. Cayambe (4575 metres) and *Erirrhinoides distinctus* Olliff. Chimborazo (4817 metres)

Very few Lepidoptera seem to occur near the snow-line on Mt. Chimborazo but the following species have been recorded at elevations of about 3962 metres *Pieris xanthodice* Luc. (2740-4575 metres) widely distributed on the Andes *Colias alticola* Godm. (Pinchincha 3657 metres, Cayambe 3962 metres, Chimborazo 3962 4575 metres Antisana west slope 4876 metres) known so far from the region only and reported by Humboldt to fly above the snow-line *Lycaena koa* Druce (3050-4267 metres) recorded also from Peru, Bolivia and Ecuador *Lymanopoda tener* Hew (3050-4400 metres) and *Pedaliodes* sp. (3050-4267 metres)

Hawaiian Mountains —Particular reference may be made here to the remarkable Carabid *Mecyclothorax caecus* Sharp with greatly reduced compound eyes, lacking pigmentation and distinct facetting occurring on the high

plateau of Kauai Island (Hawaii) Other high altitude Carabidae include *Mecyclothorax pusillus* Sharp *Mecyclothorax micans* Blackb and *Trichothorax apicalis* Sharp found at elevations of 2740-3050 metres on Maui (Haleakala) The interesting family Proterhinidae, comprising minute apterous beetles, measuring 1 5-5 5 mm long, with the head produced beak like weakly in the male and strongly in the female, occurs at an elevation of 2740 metres on Hawaiian mountains.

High Altitude Insects from the Temperate Zone of the Old World

The Himalayan System.—The Himalayan System comprises the Himalaya* The Trans-Himalayan Zaskar Ladakh Karakoram and Kailas Ranges the Alai-Pamir the Tibetan Plateau and the Kuen Lun Mountains in the north and the mountains of Persia and Afghanistan in the west. It is also continued in the east into the mountains of Upper Burma. The Himalaya proper consists of a series of converging or nearly parallel ranges of Tertiary mountains stretching east-west nearly 3000 kilometres long, from Upper Burma in the east to nearly the eastern border of Afghanistan in the west (approximately between 72° and 91 E L and 27° and 36° N L.) The width of the Himalaya is extremely variable and is in places as narrow as only 80 kilometres but in other places more than 300 kilometres. While the Himalaya rises nearly abruptly from the Indo-Gangetic plain of India in the south, it is continued in the north as a series of folds of which the Kuen-Lun represents the northernmost range of a mighty elevated surface of the earth. Between Kuen-Lun and the Himalaya proper lies the bleak arid plateau of Tibet at elevations between 4750 metres and 4880 metres above mean sea level.

The ranges that constitute the Himalaya proper are the Sivaliks the Lesser Himalayan Ranges like the Dhauladhar and the Pir Panjal and the Great Himalaya (the main range) The Sivaliks represent the southernmost zone of low hills of about 8 kilometres to nearly 48 kilometers width and separated from the Lesser Himalayan Ranges in some places by valleys called duns. North of the Sivaliks is a zone of about 80 kilometres width, with longitudinal mountain ranges parallel to the main range and rising to a mean elevation of 3000 metres above mean sea level. North of this and of about 16 kilometres width is the zone of the Lesser Himalayan Ranges, with the peaks rising to elevations of about 4575 metres above mean sea level. The fourth zone is the Great Himalaya, nearly 24 kilometres wide with perpetual snow and with numerous peaks rising to elevations above 6000 metres and sometimes also exceeding 8000 metres above mean sea level. Finally in a fifth zone about 40 kilometres wide, lie the troughs of the trans-Himalayan rivers and mountains rising to a mean elevation of 5790 metres. Some of the largest and longest glaciers in the world outside the polar area are found on the Himalaya and the Karakoram ranges

* It may be noted that it is *the Himalaya* and *not* the ungrammatical, but unfortunately widely and quite erroneously used Himalayas.

Recent explorations[35] show that the typical high altitude insects are abundant mostly at an elevation of about 4200 metres, but the highest elevation at which insects occur on the Himalaya is 6000 metres, both in the region of Mt. Everest in the east and in the region of Mt Nanga Parbat in the extreme west end of the Himalaya proper The greatest bulk of the species occurring above the timber line on the Himalaya are Palaearctic forms, belonging to the Turkmenian Subregion and only about 3% of the total insect fauna above timber line are Indo-Malayan elements. Over 75% of the species are endemic to the Himalaya Karakoram Pamir area and there are also a number of interesting endemic genera. Mani[35] has recently shown that this endemic element evolved from an original lowland stock of Central Asiatic ancestry

Ephemerida, Plecoptera and Trichoptera are extremely abundant in the melt water torrents nearly up to the snow line (5200 metres) *Rhabdiopteryx lunata* Kimm. is a stonefly that occurs, for example, at 5000 metres. Some species of *Capnia* are apterous. *Typical Orthoptera above the timber-line on* the Himalaya belong to *Bryodema* Fieber *Gomphomastax* Brun endemic to the Central Asiatic mountains *Conophyma* Zub an interesting genus of apterous grasshoppers also endemic in Central Asia and abundant on Pamir and North-West Himalaya, *Dicranophyma* Uvarov *a genus endemic to the Himalaya and* *Sphingonotus* Fieber The Tettigonid *Hypsinomus fasciata* Uvarov occurs at elevations between 4575 and 4875 metres The Dermaptera found above timber-line belong to *Anechura* Scudd. with close affinity to the species occurring on Alai-Pamir mountains. *Some extremely interesting high altitude Heteroptera* are endemic in the Himalaya Karakoram-Pamir area. Among them are two endemic genera *Dolmacoris* Hutchinson and *Tibetocoris* Hutchinson, usually found at elevations between 4000 and 5000 metres. *Nysius ericae* (Schill.) is *also found up to an elevation of 5200 metres.* The order Coleoptera is among the dominant high altitude insects on the Himalaya and represents almost one half the total insect fauna above the timber line The dominant families are Carabidae, Staphylinidae, Tenebrionidae and Curculionidae. There are several species of *Carabus* Linn and *Cychrus* Fabr in the upper reaches of the forest belt on the south slopes and some of these are also occasionally found above the timber line.

The *commonest* Carabidae above timber line belong to the genera *Bembidion* Latr *Amara* Bon. *Carabus* Linn., *Cymindis* Latr *Calosoma* Weber *Nebria* Latr *Harpalus* Latr *Trechus* Clairv *Anchomenus* Bon. *Pheropsophus* Sol., *Calathus* Bon., *Bradytus* *Clivina* Latr *Broscus* Panz., *Chaetobroscus* Sem. *Chlaenius* Bon., *Dyschirius* Panz *Leistus* Fröl *Tachys* Steph., etc *Carabus boysi* Andr occurs at an elevation of 4115 metres on the Great Himalaya and *Carabus wagae* Fairm at an elevation of 4420 metres. *Amara brucei* Andr and *Bembidion nivicola* Andr occur at an elevation of 5030 metres *Chaetobroscus* Sem is an endemic genus found at an elevation of 4000 metres. *Storeanus* Jeannel, a

genus closely related to *Trechus* Clairv., is reported from an elevation of 3050 metres on the eastern Himalaya. The Carabid genera *Hypsinephus* and *Calpodes* are absent on European mountains but on the Himalaya they are represented at elevations of 2450-4570 metres. Many Dytiscidae and Hydrophilidae are found in the glacial lakes hot springs and melt water torrents at elevations ranging from 4000 metres to nearly 5000 metres. Among the Staphylinidae, the common forms belong to *Atheta* Thoms. *Aleochara* Mannerh. *Geodromicus* Redt., *Lesteva* Latr., *Ocyusa* Kr *Oxypoda* Mannerh *Philonthus* Curt. *Pseudocypus* Cam. and *Tachinus* Gr The highest altitude record of permanent existence of Coleoptera in the world is held by a remarkable Staphylinid *Atheta* (*Dimetrota*) *hutchinsoni* Cam., which occurs at an elevation of 5600 on the North West Himalaya Many species of Tenebrionidae like *Ascalaroides cinnamilis* Bates, *Cyphogenia plana* Bates *Myatis quadricollis* Bates and *Bioramix* Bates are found at elevations between 3500 metres and 5580 metres Several species of *Laena* Latr occur at high elevations this genus is restricted to the forest zone on the European mountains, but on the Himalaya the species occur near the snow-line. *Laena alticola* Blair occurs, for example at an elevation of 5020 metres near the Rongbuk Glacier in the area of Mt. Everest. According to Blair[11] several Tenebrionidae occur at very high elevations on the Himalaya *Ascelosodis* Redt. was, for example, taken at an elevation of 5180 metres in the area of Mt. Everest. *Blaps himalaica* Blair ranges from an elevation of 4570 metres to 4880 metres and *Platyscelis aenescens* Blair between 3660 and 5030 metres Some interesting high altitude Chrysomelidae like *Chaetocnema alticola* Maulik (4000 metres) and the endemic genus *Apakshia* Maulik (3500 metres) are apterous *Leptosonyx* Weise, a genus widely distributed in Central Asia, Caucasus Siberia and Mongolia is represented by *Leptosonyx octocostatus* Weise at an elevation of 4575 metres. The Curculionids *Blosyrodes* Jekel, *Catapionus* Schönh., *Lagenolobus* *Otiorrhynchus* Germ., etc., are also abundant at elevations between 3000 metres and 4500 metres the genus *Otiorrhynchus* Germ. is however confined to the North-West Himalaya only A number of interesting high altitude ants like *Formica* (*Serviformica*) *picea* Nyl. (endemic) *Cataglyphis* (*Monocombus*) *cugei* Menozzi and bumble-bees like *Subterraneobombus melanurus subdistinctus* (Richard) and *Lapidariobombus separandus* Vogt., have also been recorded from above the timber-line on the Himalaya and Karakoram. Nearly 45% of the Lepidoptera found above the timber line on the Himalaya are endemics *Papilio machaon* Linn. is represented by many local subspecies, some of which like *Papilio machaon sikkimensis* have been found at an elevation of 5020 metres on the eastern Himalaya The greatest bulk of the typical high altitude species belong to *Parnassius* Latr., with many local subspecies of *Parnassius acco* (Gray) *Parnassius hardwickei* Gray *Parnassius delphius* Eversm., *Parnassius epaphus* Oberth. *Parnassius simo* Gray *Parnassius jacquemontii* Boisd. *Parnassius tulzchanus* Feld. etc. Some of these butterflies occur habitually at an elevation of 5800 metres on the Himalaya. The common Pierids belong to *Baltia* Moore, never found below an elevation of 3900 metres *Aporia* Hübn., *Colias* Fabr with many subspecies of *Colias electo*

(Linn.) and *Colias eogene* Feld *Argynnis* Fabr *Parnassius* Moore, *Argestina* Riley *Erebia* Dalm *Karanasa* Moore, etc., are among the other Lepidoptera often found up to 5500 metres. Diptera are extremely abundant but are at present very imperfectly known The most outstanding Nematocera are undoubtedly the mountain midges of the family Deuterophlebiidae represented by several species on the North-West Himalaya, Central Asia Altai, Formosa, Japan, Canada and parts of the United States of America. Their larvae are remarkable for their peculiar anchoring appendages and occur in icy-cold melt water torrents. The adults are curiously like mayflies in general appearance and are short-lived. Numerous Collembola are extremely abundant from the timber line to over 6000 metres on moss, lichen in soil on snow and glacier and in various other situations Several genera like *Entomobrya* Rond., *Folsomia* Wil., *Hypogastrura* Bourl. *Isotoma* (Bourl.) Börn., *Isotomurus* Börn., *Orchesella* Templ., *Onychiurus* Gerv *Proisotoma* Börn. and *Tomocerus* Nic. are represented above the timber line throughout the Himalaya

A number of high altitude insects, especially Coleoptera, ants and butterflies have been collected by the Italian Karakoram Expeditions from the Karakoram; most of these species are however also common to the North-West Himalaya [14, 23, 29, 30, 34, 61]

The Alai-Pamir.—Our knowledge of the high altitude insects from the Alai-Pamir and other Turkestan mountains is mainly due to the labours of the German-Russian, Italian and the Russian Pamir Expeditions ([1, 4, 6, 9, 16, 20, 21, 23, 33, 39, 53, 61, 65, 69, 71, 75, 80]) Some remarkable Orthoptera like *Sphingonotus* Fieber occur at an elevation of 3750 metres on the Pamir and *Conophyma zubowski* Uvarov on Trans Alai at an elevation of 3200 metres. Kiritschenko[39] has recorded a number of interesting Heteroptera from the region. Of particular interest is the genus *Mimula* Jak. a typically high altitude Asiatic form, with several species localized at elevations ranging from 2500 metres to 4300 metres on the mountain chains of Pamir Alai, Tian-Shan and Tarbagataj The genus *Myrmecophyes* Fieb is characteristic of the Turkestan mountains, but some species also occur on Caucasus and Mt. Elbrus. Altogether 28 species of Heteroptera are recorded by the Expedition from Pamir proper at an elevation of about 4000 metres There are also three endemic forms like *Mimula nigrita* Jak. *Mimula mavrei* Jak. and *Chilocoris pulai* Kiritsch *Mimula nigrita* Jak occurs at an elevation of 3000 metres, *Mimula kohlbechi* Kiritsch. at an elevation of 3400 metres and *Mimula anthracina* Kiritsch. at an elevation of 3770 metres. *Myrmecophyes oriolaris* Kiritsch. was found at an elevation of 2800 metres. *Rhinocoris monticola trochantericus* Reuter occurs from 2700 to 3800 metres. Lepidoptera from the Alai-Pamir have been described by several workers like Alphéraky[1], Grum-Grschimailo[71, 29] Avinoff[9] Filipjew[23] Rosen[71] and others. The typical Central Asiatic mountain species, which are endemic to the Alai-Pamir include *Melitaea minerva* Stgr *Erebia radians* Stgr., many species of *Satyrus* Fabr

Gonepteryx (?) Ev *Titania margelana* B B. etc. *Papilio machaon* Linn. was collected at an elevation of 4300 metres on the Pamir *Baltia shawii baitalensis* Moore and *Vanessa cardui* Linn. on the Fedschenko Glacier on Pamir at an elevation of 4500 metres, *Colias erate* Esp at an elevation of 3000 metres *Argynnis pales generator* Stgr at 3955 metres *Celerio zygophylli* Och. on the Fedschenko Glacier at 4500 metres *Bryophila sublitterata* Filip., which also extends to the North-West Himalaya was found at an elevation of 2200 metres on Shungnan. Many dark coloured species of *Erebia* Dalm. and brightly coloured species of *Colias* Fabr and large numbers of species of *Parnassius* Latr are common at high elevation on the Alai ranges and occur at elevations ranging from 3350 metres to nearly 4000 metres. The alpine zone on the Alai mountains does not descend below an elevation of 2740 metres and often also lies at an elevation of 3050 metres and in comparison to the Alps is thus rather high On the Kounjout its lower limit is at an elevation of 3960 metres. Most butterflies therefore occur over wide limits of altitudes. *Pieris chloridice* Linn occurs, for example from 900 metres to 3350 metres *Lycaena ferghana Lycaena zephyrus Argynnis lathonia* and several Satyridae occur at elevations from 600 metres to 2130 metres. *Parnassius delphius* Ev *Parnassius actius Parnassius discobolus Parnassius mnemosyne Parnassius romanovi, Parnassius rhodius Colias regia Colias hyale, Colias romanovi Colias alpherakyi, Lycaena amor Argynnis pales Melitaea fergana, Pyrgus alpinus Pieris callidice orientalis.* etc. occur on the Alai mountain chain from 900 metres to over 4880 metres on the Kounjout. Skorikow[88] records a number of mountain autochthone Bombidae like *Mendacibombus makarjini* (Skor) at elevations of 2600-3600 metres, *Sibiricobombus semenoviellus* (Vogt) at 3600-4600 metres and *Sibiricobombus morawitzi* (Rad.) at 3300-4600 metres. *Subterraneobombus* Vogt reaches nearly to an elevation of 3960 metres. Some genera like *Lapidariobombus* Vogt. *Pyrobombus* Vogt *Mendacibombus* Skor etc. are common to the Alps Pyrenees, Caucasus, Alai Pamir and the Himalaya *Subterraneobombus* Vogt and *Sibiricobombus* Vogt found on Caucasus and the Alai Pamir and the Himalaya, are not represented on the Alps and Pyrenees

The Tien-Shan mountains.—The mountain autochthone Coleoptera from the Tien-Shan Range and other neighbouring mountains include several genera like *Carabus* Linn., *Nebria* Latr., *Trechus* Clairv., *Bembidion* Latr *Pterostichus* Bon., *Lacus* Latr., *Otiorrhynchus* Germ. ect. which are mostly familiar to us as typical high altitude types from the Pamir Himalaya and the European mountains Many species like *Carabus (Ophiocarabus) politus* Mor (Tien-Shan, 2440-2740 metres) *Carabus (Ophiocarabus) aeneolus* Mor (Almatinka Pass, 3050 metres) *Carabus (Cratocechenus) elisabethae* Sem. (Ala-Tau, 1700-2800 metres) have been recorded by Semenov[78]

Mt. Fuji.—The earliest collections of insects from Mt Fuji were made by Abbe David and Matsumura[4] The latter author has recorded many species, which are peculiar to high elevations on this volcanic mountain in Japan At elevations above 1220 metres there are very few high plants and

the ground is usually covered by volcanic ash. In the zone above this elevation Matsumara found *Argynnis paphia*, *Argynnis nerippe*, *Musca domestica*, *Musca cervina*, *Leucorrhina fujiana* and *Calosoma mikado* (under stones).

The Caucasus and Ural mountains.—The high altitude insects of the Caucasus are typically endemic. In addition to the species already mentioned as having been found in the Caucasus, special reference may be made here to some high alpine Orthoptera like *Schizonotus crassicercus* Tarb. *Semenovites specularis* F. W. *Semenovites inflatus* Uvarov. *Polysarcus zalkindi* Stschelk. and *Decticus verrucosus annaelisae* Ramme, known at elevations of about 1800-3000 metres[43]. The mountain autochthone insects of the Ural mountains are very inadequately known at present and there is considerable doubt about the exact altitudes of their occurrence. The boreo-alpine species of Caucasus are *Amara erratica* Duft., *Amara quenseli* Schönh. *Geodromicus globulicollis* Mannerh. *Arpedium brachypterum* Grav. *Hypnoides rivularius* Gyll., *Eudinus interrogationis* Linn. (Coleoptera). *Pieris callidice* Esp., *Melitaea iduna* Dalm., *Agrotis fatidica* Hübn. *Plusia hochenwarthi* (Hohenw.) *Larentia munitata* Hübn., and *Gnophos myrtillatus* Thunb. (*vide infra* boreo-alpine insects).

European mountains.—The principal older mountains of Europe include the Bohemian Mass, the Erzgebirge, the Harz Mountain, the Vosges, the Central French Plateau and the mountains of Belgium, Great Britain and Scandinavia. The Carpathian, the Alps, Apennine, Kalkgebirge of north Sicily and the Pyrenees belong to the Tertiary mountain system.

The greatest bulk of the typical high altitude insects, so far known from Europe, belongs to the Tertiary mountain fauna. The roots of this are to be sought in an earlier fauna, which had evolved on the older mountains. The similarity of the Tertiary mountain fauna of Europe to those of the older mountains of Asia as far east as Siberia, suggests westward migrations from the Angaranland to the European and west Asiatic younger mountains. In the present-day insect fauna of the Tertiary mountains of Europe we may observe the undoubted evidence of the contributions from older mountain fauna. The Ostalps and the Carpathian ranges are closely related together faunistically since the present-day fauna of these two ranges have been derived from the same older Bohemian Mass and the Sudeten on one side and from the Central Mass of the Balkan Peninsula on the other side. On the other hand, the high altitude insect fauna of the West Alps is radically different from that of the Ostalps, since the West Alps was populated by stock from the central plateau of France and to a much lesser extent from the Tyrrhenian mass by way of the North Apennines. The fauna of the Spanish mountains and also of Pyrenees are sharply different. The Apennines are characterized by a mixed high altitude insect fauna which had received contributions from the Tyrrhenian, the Balkan Peninsula (over the Adriatic bridge) and also species from the Alps. The high altitude insect fauna of the mountains of the Balkan Peninsula, which exhibits bewildering variety, is undoubtedly related to

those of the Caucasus and Asia Minor On Jaila Dagh we find for example, many Caucasian elements.

Within the upper reaches of the forest zone on the Alpine System we come across a remarkable group of insects at elevations between 1100 1500 metres, some of which descend to lower elevations in local protected areas Above these limits we find species, the greatest bulk of which are wholly absent at lower elevations. The species which are typical inhabitants of the upper reaches of the forest zone in the Ostalps are *Carabus fabricii* Panz. *Carabus silvestris* Panz., *Leistus nitidus* Duft, *Nebria dejeani* (Dej) *Nebria fasciatopunctata* Mill. *Trechus limacodes* Dej *Trechus constrictus* Schaum., *Trechus grandis* Gglb., *Trechus alpicola* Sturm. *Trechus rotundatus* Dej *Pterostichus unctulatus* Duft *Pterostichus subsinuatus* Dej *Pterostichus illigeri* Panz., *Pterostichus cognatus* Dej *Pterostichus justusi* Redtb *Pterostichus schaschli* Chaud. *Pterostichus jurinei* Panz., *Enconnus similis* Wse. *Enconnus carinthiacus* Gglb *Orina intricata* Germ. *Orina gloriosa* Fabr., *Orina alpestris* Schumm. *Crepidodera cyanescens* Duft, *Crepidodera crampensis* Kutsch etc. These species are really the subalpine forms that occur in the upper reaches of the forest belt, but are also found at higher elevations to some extent. In the zone immediately above the timber-line we find a wholly new type of forms especially on some of the peaks rising to about 100-150 metres above the forests. The subalpine types decline rapidly above the timber line, but some of the forest types are occasionally found here also. It is only above an elevation of about 200 metres above the forest zone that we first come across the lower boundary of the typically high alpine zone, characterized by many peculiarities in the Alps. There are several subgenera like *Oreonebria* Dan. *Leirides* Putz. *Leiromorpha* Gglb etc. which are exclusively high alpine types. Holdhaus and Deubel[41] have listed over 75 species of Coleoptera which are typically exclusive to this zone in the Ostalps. The following are some of the more typical high alpine Coleoptera

Carabus alpestris Sutrm *Cychrus schmidti* Chaud. *Nebria fontinalis* Dan *Nebria bremii* Germ., *Nebria germari* Heer *Nebria hellwigi* Panz., *Nebria castanea* Bon *Nebria atrata* Dej., *Nebria diaphana* Dan *Broscosoma baldense* Putz., *Trechus regularis* Putz. *Trechus ovatus* Schaum. *Trechus elegans* Putz. *Trechus transfimbatus* Dan *Trechus kempeni* Gglb., *Trechus glacialis* Heer., *Trechus wagneri* Gglb *Trechus pallidus* Gglb *Trechus rudolphi* Gglb. *Trechus ochreatus* Dej., *Amara cuniculina* Dej *Amara alpicola* Dej *Amara uhl gi* Holdh. *Amara spectabilis* Schaum., *Amara nobilis* Duft., *Pterostichus ziegleri* Duft. *Pterostichus panzeri* (Panz.) *Platynus tirolensis* Dan. *Absidia birnbacheri* Kraus, *Podistra rupicola* Kiesw *Orina frigida* Wse. *Phytodecta nana* Suffr., *Crepidodera simplicipes* Kutsch., *Otiorrhynchus pictilarsis* Stierl., *Otiorrhynchus caderiens* Dan *Otiorrhynchus balcous* Stierl., *Otiorrhynchus pigrans* Stierl. *Otiorrhynchus mülleri* Stierl., *Otiorrhynchus hadrocerus* Dan *Otiorrhynchus ostipennis* Rosh., *Otiorrhynchus auricapillus* Germ., *Otiorrhynchus alpicola* Boh. *Otiorrhynchus tridentatus* Dan., *Dichotrachelus tirlisi* Gredl., *Dichotrachelus knausi* Pen. *Dichotr*

pygmaeus Sterl., *Aphodius hilimeki* Seidl *Aphodius pollicatus* Er., *Aphodius montivagans* Er *Aphodius praecox* Er In parts of the Alps where glaciers exist at present, the species ascend higher nearly up to the snow line. Many high alpine species of *Nebria* Latr., like *Nebria atrata* Dej on the Hohen Tauern, *Nebria brevis* Germ. on the Swiss Alps exist only in the higher reaches of the high alpine zone and their lowest limit of occurrence is 200-300 metres higher than the lower limit of the other high alpine beetles. Thus *Nebria atrata* Dej. occurs on the eastern Hohen Tauern only at elevations of 2400-2500 metres, while the rest of the high alpine species like *Nebria castanea* Bon. *Nebria exstincta* Gglb., *Nebria hellwigi* Panz *Nebria germari* Heer etc are restricted to zone of about 2150-2200 metres above mean sea level. *Nebria atrata* Dej., and *Nebria brevis* Germ are typically subnival elements.

The most important contributions to the knowledge of high altitude insects of the Swiss Alps include those of Bäbler[16] Franz[17] Holdhaus[24,30] Handschin[24,34] and Janetschek[44] At an elevation of about 2800 metres on the south slope we find under stones the caterpillars of *Bryophila tenebraria* Esp *Psodos alticolaria* Mn. *Gnophos caelibaria spurcaria* Latr *Gnophos zelleraria*, *Nebria brevis* Germ. *Isotoma alticola* Carl. and *Isotoma nivalis* Carl. On the north-west slope at about the same elevation we also find *Bombus hyperboreus* Linn. At elevations of 3050-3230 metres are found *Bembidion glaciale* Heer *Cymindis vaporariorum* Linn., *Erebia glacialis* Esp *Vanessa urticae* Linn., *Psodos alticolaria* Mn., *Isotoma saltans* Nic. etc. Numerous Diptera and mites have been recorded at higher elevations near 4000 metres

The general climate of the Carpathian range is far more continental than that of the Alps and the timber-line is lowest in the north but high both in the east and south Carpathian. On the Babiagora the timber-line is at an elevation of 1300 metres, in the Central Carpathian (Hohe and Nieder Tatra) at 1500 metres, in the Rodnaer Alps and Rareul at 1600 metres, in Kelemenstock, the Hargita and Nagy Hagymus at 1700 metres and in the south Carpathian it lies between 1700 and 1800 metres. In the Transsylvanian Alps the timber line is about 200-300 metres higher on the south slope than on the north slope. We have an excellent review of the high altitude insects, especially the Coleoptera, from the Carpathian by Holdhaus and Deubel[41] Comparing the salient features of the high altitude insects of the Alps with those of the Carpathian we find that while the high alpine zone of the Alps is rich in Coleoptera, that of the Carpathian is on the other hand relatively poor in exclusively high alpine Coleoptera. We know for example, at least 80 species of typically and exclusively high alpine Coleoptera from the Ostalps, but there are no more than 20 such on the Carpathian and most of them also often occur at much lower elevations We do not also find on the Carpathian exclusively high alpine species of *Cychrus* Linn., *Carabus* Linn., *Trechus* Clairv., *Amara* Bon. *Orina* Motsch *Crepidodera* Chevr., *Leptusa* etc. The subgenus *Oreonebria* Dan. of *Nebria* Latr so characteristic of the Alps is also absent. The Coleoptera from the Carpathian differ also from

those of the Alps in the remarkable predominance of the taiga species and the comparative poverty of the typically and exclusively high alpine elements. The Ostalps has on the other hand only a few of the taiga insects and these are also confined on the whole to the lower reaches of the high alpine zone. The exclusively high alpine species of the Ostalps belong to genera like *Carabus* Linn., *Nebria* Latr., *Trechus* Clairv., *Amara* Bon., *Pterostichus* Bon *Orina* Motsch *Crepidodera* Chevr *Otiorrhynchus* Germ *Dichotrachelus* *Aphodius* Ill. etc. The Carpathian is, however characterized by the total absence of the *castanea* group of the subgenus *Oreonebria* Dan. of *Nebria* Latr so very typical of the high alpine zone of the Ostalps. We do not also find here the *pertyi*-group of species of *Trechus* Clairv and the subgenera *Euromorpha* Gglb and *Leirides* Putz of *Amara* Bon and other familiar high alpine *Carabus* Linn. *Cychrus* Linn., *Orina* Motsch. *Crepidodera* Chevr and *Dichotrachelus* The number of exclusively high alpine species of *Otiorrhynchus* Germ. found on the Carpathian is indeed very small in comparison to the Alps Of about twenty exclusively high alpine Coleoptera, known from the Carpathian, *Oxypoda nimbicola* Fauv *Otiorrhynchus alpicola* Boh. and *Aphodius montanus* Er are also known from the Ostalps. Nine of them, strictly endemic to the Carpathian, viz., *Corypheodes beckeli* Bernh. *Niphetodes reditenbacheri* Gglb *Mycetoporus oreophilus* Bernh., *Chalcoa oreotropha* Gglb., *Rybinskiella magnifica* (Ryb) *Chrysomela schneideri* Wse., *Otiorrhynchus fasciventris* Fst *Otiorrhynchus hypsibatus* Gglb and *Brachyodontus rattieri* Wse have a localized distribution. Ten other species which are also endemic to the Carpathian and also occur strictly in the high alpine zone, but are widely distributed within the Carpathian include the following *Nebria carpathica* Bielz *Nebria tatrica* Mill *Leistus gracilis* Fuss *Deliomerus tatricus* Mill., *Atheta carpathica* Mill. *Niphetodes deubelli* Gglb. *Niphetodes speciosus* Gglb., *Bledophaga alpicola* Kiest. *Otiorrhynchus alpigradus* Mill. and *Otiorrhynchus granicollis* Boh

The Atlas Mountains—Among the high alpine Coleoptera fauna of the Atlas mountain we find species of various genera already known to be typical of the alpine zones of the European mountains. We have, for example, such genera like *Nebria* Latr *Trechus* Clairv *Deliomerus* Motsch. *Amara* Bon. *Zabrus* Clairv *Atheta* Thoms. *Geostiba* Thoms *Oxypoda* Mannh. *Peraleptusa* Peyerimh. *Malthodes* Kiesw *Dasytes* Fabr *Pseudoreosites* Heyd. *Timarcha* Latr *Laperus* Geoff. *Otiorrhynchus* Germ. etc. There are exceedingly few endemic mountain genera on the North West African mountains the carabid genera *Trechopsis* Peyerimh. and *Oreocys* Peyerimh. are perhaps the only typical endemic genera

Boreo-alpine Insects

The boreo-alpine elements occupy an important place among the mountain autochthone insects of Europe The boreo-alpine insects are characterized by their discontinuous distribution in the northern parts of Europe and at high elevations on the Central European and partly also south European

mountains but are absent in the intervening areas Many of the boreo-alpine species in the north extend also to Siberia in the east and to a very limited extent occur on the Altai mountains and only exceptionally rarely on the Himalaya Many of the boreo-alpine species are circumpolar in the north and thus occur in the extreme north Europe Asia and America. The grasshopper *Podisma frigida* Boh., for example, occurs in Fennoscandinavia, northern Russia Siberia, Altai, northern Mongolia, Manchuria, Kamtschatka, Alaska and the Alps Many boreo-alpine species of Coleoptera are known *Nebria gyllenhali* Schönh., *Bembidion assimilis* Chaud *Bembidion difficile* Motsch., *Patrobus similis* Chaud. *Pterostichus blandulus* Mill., *Pterostichus kokelii* Mill., *Amara erratica* Duft. *Amara quenseli* Schönh., *Trichocellus mannerheimi* Sahlb., *Mannerheimia arctica* Er *Arpedium brachypterum* Grav *Geodromicus globulicollis* Mannerh., *Anthophagus alpinus* Fabr *Atheta laevicauda* Sahlb., *Helophorus glacialis* Villa, *Podabrus obscuripes* Sahlb., *Corymbites cupreus* Fabr., *Chrysomela crassicornis* Hellies. *Otiorrhynchus arcticus* Fabr., etc. Some Lepidoptera like *Parnassius phoebus* Fabr., *Pieris callidice* Esp. *Argynnis pales* Schiff., *Erebia lapponica* Esp *Erebia epiphron* Knoch *Gnophos sordarius* Thunb., *Psodos coracina* Esp *Arctia quenseli* Payk. etc. are also boreo-alpine elements on the high altitudes of European mountains. Very few of these boreo-alpine species occur within the forest zone this is for example the case with *Erodius interrogationis* Linn. The greatest majority of the species inhabit the zones above the timber-line some of them are found in the subalpine and others in the high alpine zone. Of the forty three species of boreo-alpine Coleoptera from Europe, only *Bembidion fellmanni* Mannerh. *Bembidion difficile* Motsch *Pterostichus blandulus* Mill. *Boreophilus henningianus* Sahlb. *Otiorrhynchus arcticus* Fabr and *Barynotus squamosus* Germ. are absent on the Alps Though the Alps has the majority of the boreo-alpine species only a small number of them occur at high elevations throughout the Alps and the others are localized in the West Alps or the Ostalps as the case may be. The boreo-alpine Coleoptera of the Carpathian and the Sudeten mountains are similar Of the twenty species found on the Sudeten range, two do not occur on the Carpathian. The Carpathian has in addition *Bembidion fellmanni* Mannerh., *Bembidion difficile* Motsch., *Amara quenseli* Schönh., *Pterostichus blandulus* Mill. *Pterostichus kokelii* Mill., *Erodius interrogationis* Linn *Otiorrhynchus morio* Fabr., etc. There is thus a total of 29 species of boreo-alpine Coleoptera on the Carpathian. On the Apennine Range only *Nebria gyllenhali* Schönh., *Anthophagus alpinus* Fabr., *Helophorus glacialis* Villa and *Corymbites cupreus* Fabr occur We have on the Pyrenees *Nebria gyllenhali* Schönh. *Amara quenseli* Schönh *Amara erratica* Duft., *Geodromicus globulicollis* Mannerh *Helophorus glacialis* Villa, *Corymbites cupreus* Fabr., *Otiorrhynchus morio* Fabr *Otiorrhynchus arcticus* Fabr etc., and seventeen species of Lepidoptera like *Argynnis pales* Schiff., *Erebia lapponica* Esp *Pieris callidice* Esp *Psodos coracina* Esp., *Gnophos myrtillatus* Thunb., etc On the Spanish Sierra Nevada occurs *Helophorus glacialis* Villa. The boreo-alpine elements of the Jura Mountains are *Nebria gyllenhali* Schönh., *Amara erratica* Duft, *Anthophagus alpinus* Fabr *Geodromicus globulicollis* Mannerh., *Otiorrhynchus*

moro Fabr., etc. *Amara erratica* Duft and *Otiorrhynchus moro* Fabr are among the boreo-alpine elements of the Vosges mountains also

The boreo-alpine species are also known as glacial relicts It was generally assumed that the Pleistocene glaciations brought about whole-sale destruction of flora and fauna and the relict species were assumed to have survived in the marginal zone of the Alps, termed by Holdhaus[34 35] as "massifs de refuge At the end of Pleistocene the relict species are supposed to have rewandered to the interior of the Alps Janetschek[41] has however recently shown that the boreo-alpine species have actually survived the Pleistocene glaciations in the heart of the Alps on high peaks which remained as rock islands (nunatak) above the ice mass

High Altitude Insects from the North Temperate Zone of the New World

In Canada the Western Highlands about 600 kilometres wide, comprise the Western Mountain Range, the Middle Range and the Eastern Range. The Western Range contains the Cascade Mountains with Mt. St Elias as the principal peak, reaching up to an elevation of 5950 metres and situated in Alaska. On the Middle Range are situated the Gold Mountains and the Eastern Range constitutes the Rocky Mountains. Many ridges in the Western Highlands rise to about 3050 metres above mean sea level Between the main mountain ranges is the plateau of British Columbia. The Labrador plateau constitutes the Eastern Highlands of Canada. In the United States of America, the Western Highlands contain the Cascade Mountains the Sierra Nevada and the Rockies in the east with plateaus in between In the eastern United States the mountains are neither so high nor so extensive as in the west. The only mountains of importance n the east are the New England Mountains, the Appalachian Highlands separated by the Hudson Valley The Appalachian Highlands comprise the Allegheny Plateau in the west and the Appalachian Mountains in the middle and the Piedmont Plateau in the east. The mountains of northern Mexico lie partly in the temperate zone and comprise a western and an eastern range of the Sierra Madre with plateau in between.

Our knowledge of the high altitude insect life of the North American mountains is, surprisingly enough very fragmentary despite the fact that the science of entomology has advanced in America more than elsewhere for example Europe. The White Mountains in the Eastern USA have perhaps been better explored than most other North American mountains The highest peak in this area is the well known Mount Washington (near 44° 30′ N L.) reaching to an altitude of about 1918 metres above mean sea level but at the same time reaching to elevations above the timber line, which lies at an elevation of 1530 metres on this mountain. The insects from this mountain have been described chiefly by Scudder[73] Austin & LeConte[4]

Bowditch[14] LeConte[13] Slosson[11] and others. The following are some of the more important Coleoptera so far recorded from above the timber-line on Mt. Washington

Carabus chamissonis Fisch., *Calosoma frigidum* Kirb *Nebria suturalis* LeC. *Nebria sahlbergi* Fisch., *Nebria pallipes* Say *Trechus rubens* Fabr *Platynus bohemani* Gyllh. *Bradycellus cognatus* Gyllh *Acidota crenata* Fabr *Simplocaria metallica* Sturm in addition to several species of *Bembidion* Latr *Patrobus* Steph., *Pterostichus* Bon *Amara* Bon *Harpalus* Latr *Agabus* Leach, *Silpha* Linn., *Quedius* Leach *Corymbites* Latr *Philonthus* Steph., *Cytilus* Erichs., *Byrrhus* Latr *Hypnoidus* Steph *Leptura* Linn *Chrysomela* Linn. *Lepyrodes* Motsch., etc. We may observe a general preponderance of boreal forms, with several genera, which are also found on high mountain ranges in the Palaearctic Realm The species are largely however Nearctic, but some of the beetles found on the summit of Mt. Washington, such as for example *Trechus rubens* Fabr and *Simplocaria metallica* Sturm. are also found on Central European mountains and are generally widely distributed in the high north latitudes.

On the Rocky Mountains, at latitudes of about 56° North, the timber-line is at an elevation of about 1220 metres above mean sea level On Montana the timber line lies at an elevation of 2900 metres and at a latitude of 39° North the timber line is at an elevation of 3700 metres These generally high timber lines of the Cordillera doubtless explain the high altitudinal limits reached here by the subalpine types Carpenter[19] found for example, that the insect fauna of the Colorado mountains is very rich at an elevation of 3350 metres, that is a little above the timber-line. A few hundred metres higher still, the abundance of species falls off markedly and insects are said to be generally very scarce at an elevation of about 4260 metres. An elevation of about 2750 and 3350 metres corresponds roughly in Colorado to the subalpine zone of the European mountains LeConte[18] has reported from the Colorado mountains a number of interesting genera like *Trachypachys* Motsch *Notiophilus* Dum *Nebria* Latr *Bembidion* Latr *Platynus* Bon., *Pterostichus* Bon. *Calathus* Bon *Amara* Bon. *Harpalus* Latr *Hydroporus* Clairv *Helophorus* Fabr., *Stenus* Latr *Geodromicus* Redtb *Lathrobium* Latr., *Olophrum* Erichs *Deliphrum* Erichs. *Orobanus* LeC., *Anthobium* Leach, etc.

There are many forms on the Rockies belonging to genera which are met with at high elevations on Central and south European mountains. Among the Coleoptera found above an elevation of 3960 metres in Colorado are *Carabus taedatus* Fabr *Tachypachys inermis* Motsch. *Notiophilus hardyi* Putz., *Cymindis unicolor* Kirby *Pterostichus* (*Cryobius*) *surgens* LeC. (2750-3965 metres), *Amara gibba* LeC. *Amara* (*Curtonotus*) *cylindrica* LeC. *Aphodius leopardus* Say *Podabrus lateralis* LeC (2900-39o5 metres) *Collops bipunctatus* Say, *Asemum atrum* LeC., *Chrysomela montivagans* LeC. (3350-3960 metres) *Adimonia externa* Say *Epicauta pruinosa* LeC and *Lepyrus gemellus* Kirby Many of these species often occur within the forest zone immediately below the timber-line and it is thus not possible at present to give a list of the exclusively high alpine Coleoptera of

the Rockies. It is however extremely interesting to observe that the genus *Carabus* Linn which is widely distributed both in the subalpine and the high alpine zones on Central European mountains is rather poorly represented on the Rockies. Indeed no typical mountain autochthone Nearctic species of the genus seems to have found so far The tribe Cychrini is however represented by several species, both in the subalpine and alpine zones of Nearctic mountains, but there are perhaps no true high alpine species of the genus *Cychrus* in the region. Relatively few species of *Trechus* Clairv are known at present and none of them are also exclusively high altitude forms. The total absence of *Otiorrhynchus* Germ on the Nearctic mountains is very significant, when we recollect that this genus is found in the plains of North America, with a number of species introduced from Europe It must also be observed that the Chrysomelid genus *Orina* Motsch. is not represented on the Nearctic mountains.

The high altitude Lepidoptera from the Nearctic mountains include species of *Parnassius* Latr., *Colias* Fabr., *Oeneis* Hübn. *Erebia* Dalm *Argynnis* Fabr etc genera which are widely distributed in the Palaearctic also. While *Parnassius* Latr occurs in Alaska up to the arctic circle, in the rest of North America, it is however found on the Cordillera, southwards up to 35 N.L. and ascends here usually to an elevation of about 4000 metres. Even *Erebia* Dalm. and *Oeneis* Hübn are found at high elevations for example, *Erebia epipsodea* Butl occurs at an elevation of 3660 metres in Colorado *Erebia magdalena* Steck. at an elevation of 3050-4270 metres in Colorado *Oeneis beani* Elw at 2440-2750 metres in Alberta (Canada) *Oeneis semidea* Say and *Oeneis bruca* Edw at 3660-4270 metres in Colorado

Many grasshoppers occur at high elevations on the North American mountains The Acridid genus *Podisma* Latr found on the Himalaya and Europe, is represented in North America by a typically mountain autochthone species *Podisma nubicola* Scudd which occurs at an elevation of 3350-3970 metres and naturally above the timber-line on Mt. Lincoln Colorado. *Podisma stupefacta* Scudd occurs on the Tao Peak in New Mexico at 396* metres *Podisma dodgei* Thom at an elevation 1830-3350 metres in Colorado and *Podisma marshalli* Thom. at an elevation of 3350-3960 metres also in Colorado The genus *Melanoplus* Stal. related to *Podisma* Lat is also represented by a number of typical mountain autochthone pecies like *Melanoplus altitudinum* Scudd., occurring from Montana to New Mexico and on Taos Peak n the latter area at an elevation of 3960 metres *Melanoplus monticola* Scudd., in Sierra Blanca in Colorado above the timber line at elevations of 3660 metres and 3960 metres *Aeroloctes philopagus* Rehn & Hebard is perhaps a typical mountain locustid, which has been found at an elevation of 3960-4400 metres on Sierra Nevada, on Mt. Whitney and other parts of the southern Sierra Nevada at elevations of 3200-3660 metres.

High Altitude Insects from the Mountains of the South Temperate Zone

The principal mountain range in the south temperate zone is the southern part of the Andes. In this part of the Andes the timber line and the snow-line are naturally much lower than in the Chilean Andes and nearly arctic conditions prevail. Some high altitude insects from this are known. The high altitude Coleoptera from this part are relatively poorly known; the Lepidoptera are however somewhat better known. Most of the high altitude butterflies of the southern Andes are typically small forms and remind us of the high alpine butterflies of the Alps in their general colour and habits. The genera which are peculiar to the southern Andes are *Phulia* and *Trifurcula* Zell. which have their close relatives among the Pierids of Asia, but *Colias* Fabr and *Pieris* Linn. are also found. The Nymphalidae are here represented by *Argynnis* Fabr and *Vanessa* the Satyridae by *Pseudomaniola* *Lymanopoda*, *Pedaliodes* *Steroma* etc., in place of the boreal *Erebia* Dalm. and *Oeneis* Hübn. Among the Heterocera we find *Agrotis* O *Prodenia*, *Mamestra* *Psodos* *Arctia*, etc.

Some high altitude insects from the mountains of the Australian region are also important. The Satyrid genus *Heteronympha* Wallgr is for example typical of the high mountains of New Zealand and Tasmania. Likewise, the genus *Xenica* Butl. also contains many species which are typical and exclusively high altitude forms, like *Xenica* *muckera* Meyr and *Xenica* *correae* Olliff, which have been reported from Mt. Kosciusko at elevations of 1500-1800 metres Many species of the genus have a localized distribution on Tasmanian mountains. Some species of *Erebia* Dalm have also been reported from the mountains of New Zealand, but this genus is absent in Australia and Tasmania. *Erebiola* *butleri* Fer and *Percnodaimon* *pluto* Fer., closely related to mountain Satyridae of the Palaearctic are exclusively mountain species and occur at elevations of 1000 1850 metres on the South Island (New Zealand) Other mountain species belong to *Melacrias* Meyr., *Notoreas* Meyr *Dasyuris* Gn. etc. The adult female of the Arctiid *Melacrias* Meyr is remarkable for its greatly atrophied wings. The grasshopper *Pyrgomorpha* Burm is reported to be typical of the mountains of New Zealand

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PRENATAL DEVELOPMENT OF *BUFFALO—BOS BUBALIS* L.

SURJAN SINGH O P S SENGAR AND S. N SINGH
Department of Animal Husbandry & Dairying B R. College Agra

Buffalo—*Bos bubalis L.*, plays a significant role in the economy of several tropical countries and occupies a unique position amongst their livestock. By combining both quantity and quality in their milk they fetch more profits to their owners. Besides, they provide tillage and transport in swampy-tracts, meat to certain classes of people and excellent quality hide for our varied use. Above all, to consume comparatively poorer feeds and convert them into richer food is their speciality In India the shifting interest of the dairymen from cow to buffallo has revolutionized the traditional thinking and outlook of the people. Cow which has for long monopolised the favour of the masses under the shielded protection of so-called religious sentiment, fails to compete with buffalo as milk producer The promising venture of buffalo therefore, calls for a detailed investigation about this animal. Along with the several projects which are already under way or are being contemplated to be taken up, studies on calf raising programmes, in order to explore the productive efficiency of this animal, cannot be ignored.

It is needless to emphasize the fact that the calf raising programme does not start after the calf is born but from the time it is seeded. In order that the developing calf inside the womb may effectively be taken care of and its growing needs be met with, an attempt has been made to collect some information on the rate of foetal development in buffaloes and establish the normal growth curve with regard to gestation, so that the feeding of the females during pregnancy could be made adequate, effective and economical. This might also be of use in the veal producing programmes and help in getting heavier birth weights for more profits. In addition it will also help in pregnancy diagnosis and in evaluating the effects of varied environments on foetal growth the possibility of which is only speculative for want of sufficient information.

REVIEW OF LITERATURE

The scientific and systematic information on prenatal development of farm animals is scarce. It is altogether wanting in case of buffalo, although, some parallel information is available about pig, sheep and cattle in foreign literature. Lowrey (1911) Patten (1931) and Arey (1934) studied the embryology of pig based on slaughter house specimens, the ages for which were assigned on the basis of their linear measurements. Their observations are quite in line with those of Warwick (1928) who conducted similar studies on timed specimens. Mitchell and associates (1931) on the other hand have

(6) *Medium neck circumference*—It was taken by running a thread about the middle of the neck.

(7) *Chest circumference*—It was measured just rear to the forelegs.

(8) *Abdomen circumference*—This was taken just touching the umbilical cord.

(9) *Chest depth*—It was the vertical distance between the floor of the chest immediately behind the forelegs and the top of the withers.

(10) *Chest width*—It was the horizontal distance between the two shoulder points.

(11) *Horizontal head circumference*—It was taken across the glabella. Four pins were used, two close to the base of the ears and the other two over the eye region of the skull. A thread was then run around the head below the pins.

(12) *Head height*—It was the shortest distance between crown top and the base of the mandible.

(13) *Head width*—It was the horizontal distance between the points just infront of the ears.

(14) *Head length*—It was measured from beneath the chin to the top of the crown.

(15) *Face length*—This was a projected measurement from the line joining the inner corners of the two eyes to the point of the chin.

(16) *Height at withers*—It was taken from the point of hoof of the foreleg to the top of the withers.

(17) *Height at pin*—It was noted from the point of hoof of hindleg to the pin point.

(18) *Tail length*—This was taken from the base to the tip of the tail.

Technique of Age Determination

Assuming the nature of prenatal development in cow and buffalo to be similar the data collected by Winters, Green and Comstock (1942) for cattle have been used as the basis of age determination In order to make the estimates fairly accurate four different body measurements, i. e., pin-crown length, chest circumference, abdomen circumference and horizontal head circumference were used. The absolute values for these measurements for cows were converted into the relative values, expressed as the percentage of the standard gestation period and size. This was essential because of the initial difference between the gestation period and the size of the two species The graphs were then plotted between the stage of pregnancy (expressed as percentage of the total gestation period) and size (expressed as percentage of normal size of the foetus at the end of the gestation period) and the smooth curves drawn

The stage of pregnancy in terms of percentage of the gestation period was estimated for each individual on the basis of the different curves drawn for different measurements These percentage values of gestation period varied from one another for the same individual due to the difference in the rate of development of various body parts at different stages of pregnancy Thus in order to get the most representative curve satisfying the different body measurements, pooled average of the above values for different stages of gestation were worked out, plotted and a smooth standard curve obtained This curve from the data of Winters, Green and Comstock (1942) for cow thus served as the standard for estimating the stage of pregnancy as percentage of gestation period from the measurements of the foetuses for buffaloes. These percentages were then converted into actual stage of pregnancy in days taking the normal gestation period for the species as 310 days.

The age groups with an interval of 30 days were then formed on the basis of estimated age and the averages for the different measurements were worked out for different age groups. The graphical representations of the different average body measurements of the estimated age have also been made for comparison Normal gestation period for buffaloes was taken as 310 days while the standard body size at the end of the gestation period was based upon the average body weight of 65 lb (Average birth weight of 100 buffalo calves at Military Dairy Farm, Agra) Different body measurements of calves approching the average weight (65 lb) were taken and averaged. The standard size thus obtained for buffalo calves at birth was utilized for converting the absolute values into relative values expressed as percentage of the standard size at birth.

Some of the specimens have also been photographed against the metric graph in order to give the comparative idea about their size at various stages of development. These were taken with the help of a 35 mm. contaflex camera using close up lenses for the larger specimens, while the smaller ones were photographed under a wide field binocular These photographs have been included in support of the text.

Experimental Findings

Description of the Specimens

Reproduction amongst animals is no different from growth. It is actually the multiplication in number after the cessation of the somatic growth. Growth of the individual from the formation of the zygote (a single celled stage) until birth is a continuous process (Fig 5) However the prenatal period, for the sake of convenience, can be divided into several phases of development without altering the basic concept of continuity These phases can be designated either on the basis of different body measurements as length, weight and volume or the development of different organs or just by appearance.

The prenatal period is usually divided into three major phases namely the ovum the embryonic and the foetal based upon some of the more critical moments and the amount of development during the intra-uterine existence. As usual the period of ovum in buffaloes should last from the time of fertilization until the attachment of the blastocysts to the endometrium of the uterus. The embryonic phase is generally the period during which the genesis of different organs and systems takes place. Although most of the changes during this period are internal the body shape also undergoes a series of successive changes. The body torsion characteristic of the embryo at early stages is lost and the specimen assumes a rather C-shaped structure. This too is altered at later stages by the appearance of rather definite points of flexure. These and the other changes in body shape make it possible to describe embryonic development not only in terms of linear measurements, but also in terms of body outline. As the genesis of the organ system is almost complete during embryonic period itself the main changes that occur during the foetal stage are those of growth and minor details of differentiation. The point of demarcation between the embryonic and the foetal stage is somewhat arbitrary However the duration from 50 days to the end of gestation has been termed as the foetal period, mainly because all body organs get well differentiated by about the 50th day In case of cattle, on the other hand, organo-genesis is complete within 45 days as indicated by the studies of Winters, Green and Comstock (1942) This is probably due to the comparatively more rapid rate of development in cattle than in buffalo.

Period of Ovum

The phase of ovum could not be studied for want of proper facilities of recovering the ova from the females and the recorded pregnancies.

Embryonic Period

The embryonic period as usual begins at the time the embryo attaches itself to the uterine wall. A close examination of the embryonic attachment revealed that in this case it does not bury itself into the uterine mucosa as is the case with primates and rodents. It only makes a surface to surface contact with the wall of the uterus at the specialised points called cotyledons as in case of cow and sheep. A series of embryos has been shown in Figs. 6, 7 and 8 The early ones (Fig 6) which did not establish placental contacts with the uterus could be easily gathered by flushing the gravid uteri with normal saline while the older ones (Fig 8) had to be detached from the uterine mucosa at cotyledons which develop progressively

As has already been indicated the very early embryonic stages also could not be given due attention for want of adequate facilities. The youngest specimen (about 28 days old) has been shown in Fig 9 It has a C-shaped structure with a contour length of about 1 5 cm. and weighs approximately 0 06 gm. The branchial clifts and their accompanying arches are clearly noticeable

as oval slits just behind the cephalic bulge The optical vesicle is quite prominent and the otocysts, heart, liver and mesonephric regions are also quite distinct. All somites are almost formed and the tip of the posterior region is still blunt and does not grow into the tapering tail. The remnants of the yolk sac along with the progressively developing allantois are distinctly visible.

As the embryo develops further few characteristics like addition in the branchial arches, reduction in the heart bulge, large mesonephric and liver prominences and a little more differentiation of the head region along with the eye-spots get noticeable. The limb buds start making their appearance and in about 32 days old specimen (Fig 10) they become quite prominent. Of the two limbs anterior ones seem to make a quicker progress in growth than the posterior ones. At this stage the contour length changes to about 2 2 cm. and the weight increases to about 0 14 gm. The mesonephric, liver and the limb prominences increase progressively with the growth of the embryo. The blunt posterior region starts turning into a pointed tail.

By about 36 days the weight increases to about 0.2 gm. with a contour length of 3.3 cm. The body at this stage also maintains its C-shaped disposition without significant differentiation in the facial features (Fig 11) The branchial arches reduce while the nasal pits ear slits and eye spots get clearly defined. The heart bulge reduces, while the liver prominence increases considerably to fill the concavity of the embryo almost completely The limb outgrowths continue to grow as the embryo enlarges in size and assume somewhat clubbed and flattened appearance.

By about 40 days of gestation the embryo starts increasing rapidly on its ventral side as a result of which the C-shaped contou of the body is lost and it gets linearly extended. At this stage pin to crown length is about 1 4 cm. The embryo weighs about 0 49 gm. and the mesonephric region becomes much more prominent, while the heart bulge reduces significantly (Fig 12) At this stage the head starts elaborating eye and the lower jaws become prominent and the slit representing the future mouth gets clearly established. Limbs along with tail continue to grow further and the former assume a flipper like appearance

As the embryo grows further there is an extension of the abdomen due to the liver enlargement. The head which was so far laterally compressed gets somewhat rounded with a conical cephalic prominence on its top The eyes get prominently defined and start sinking into the head with eyelids developing around them. The ear slit gets restricted to a pit like structure without external ear (pinna) The umbilical cord gets prominently developed and the genital tubercle growing just below the tail also gets visible. But elaboration of the sex is not clear externally The limbs start developing their joints and the extremities start differentiating into the future hooves.

By about 45 days of gestation (Fig 13) when the embryo weighs about 0·77 gm, and measures about 1 7 cm. from pin to crown limbs develop further with elaborating hooves and dewclaws. The fore limbs precede the hind limbs as far as development of these features is concerned Sex differentiation also starts from this point onwards. The cephalic dome nearly approaches its maximum growth at this stage, and then progressively regresses with the growth of the embryo The face which was almost flat so far starts growing into a snout, the eyes start sinking and the ear slits get rounded up into open pits without pinna or external ear

By about the 50th day of gestation (Fig 14) the sex gets clearly differentiated The tip of the genital tubercle turns and points backwards if it were to be a female foetus whereas in case of a male it runs forwards upto the ambilicus close to the abdominal wall The labio-scrotal swellings (genital swellings) also make their appearance as small domes one on either side of the genital tubercle as early as about 45 days of gestation. These domes progressively increase in size, migrate posteriorly due to the straightening of the ventral line of the foetus and come to fuse with each other forming the scrotal pouch by about the 80th day of gestation in case of male foetus. These domes, which also appear in female foetus to start with, elaborate for a while and then regress progressively with the growth of the foetus and probably get incorporated with the developing vulval lips

The liver prominence is about the maximum of its development at this stage. The limbs start elaborating their joints, points, dewlaws and hooves. The face gets snouted and provided with well differentiated lips tongue and nostrils. The ear openings gets covered by the growing external ear lobe and the eyes develop well defined lids around them. The cephalic dome on the top of the head regresses, but the enlarged cerebral hemispheres account for a prominent bulge anteriorly At this stage the specimens measure about 2·4 cm. between pin and crown and weigh about 1 66 gm

Thus the 50th day stage is the stage wherein almost all the body organs have laid their blue prints. This can, therefore be safely regarded as the transitional stage between the embryonal and the foetal phases.

Foetal period

About 84% of the total gestation period in case of buffaloes is occupied by the foetal phase, which is in close agreement with that for cattle The major changes in the foetus at this stage are of growth and continuing differentiation. The eyes get partially covered with the growing lids by about 65 days of gestation (Fig 15) and are covered completely within a few days afterwards (Fig 16) Some of the important changes in the body form which take place during this period are associated with the alterations in the head and the neck regions These are the reduction in the size of the cephalic dome a recession of the cervical flexure further elaboration of facial features,

elongation of the neck and the orientation of the head at 90° with the longi tudinal axis of the body Figures 15 to 20 help better than words in gaining a better conception about the changes the specimens undergo at this phase of development The specimens referred to above also reveal a few abdominal changes like the reduction in the size of liver prominence and an extention of the body wall to the umbilicus. The ribs, laid down during embryonic period, start becoming prominent due probably to the ossification of their vertebral portions.

The appendages, in general, undergo an interesting change The short and thick embryonic limbs progressively elongate and elaborate with regard to different joints, points and outline till birth. However the hind limbs which have been lagging behind during the embryonic phase assume a rela tively quicker development and approach the front ones not only in length but also in the degree of differentiation.

The mammary glands, as conspicuous white spots, just below the skin, a pair on either side of the mid ventral line, make their appearance by about 65 days of gestation. In males these spots lie on an arc whereas in females they are more or less squarely placed. The spots change to mammary hillocks at about the 80th day of gestation. The arc in case of males straight ens with foetal growth so much so that it forms almost a straight line in a 150 day s foetus Of the two sexes, the females, in general, seem to make a quicker progress in respect of mammary glands than males. In females they are a little more conspicuous with better developed and placed teats than their counter part in males. Their further growth is almost isometric i e propor tional to the general body growth.

Some of the fundamental changes in body proportion mentioned above continue to take place till about the 100th day of intra-uterine existence. The alterations in the body from 100th day onwards till birth, though definite, are not radical, as can be seen from Figs. 17 to 24 Although genetic make-up of the parents is said to be responsible fo the slight differences in skin and hair pigmentation, body proportions and foetal size at a given age of the individual, it apparently does not significantly affect the general trend of the body changes in foetuses.

A few features in addition to the body measurements, may also assist in estimating the stage of pregnancy Horn pits make their appearance for the first time at about the 250th day of gestation and elaborate at a very slow rate, so that the horn buttons are formed by about the 275th day and grow into small prominences by the time of parturition. Pigmentation of the skin on the other hand apparently starts from about the 150th day continues progressively and is completed by about the 200th day of gestation. A few small hair make their appearance over the muzzle and eye lids at the age of about 175 days and by about 200 days the hairs also appear on eyebrows,

but the hair-coat does not cover the body until approximately 275 days of gestation

PRESENTATION OF MEASUREMENT DATA

The average values for different foetal measurements at monthly intervals have been presented in Table 1 along with those of the newly born calves and represented graphically to show their general trend.

The average body weight and volume when plotted on estimated age (Graph 1) give a fairly smooth curve. It clearly indicates that the gross foetal development is very slow in the beginning and then shoots up suddenly after about 165 days of gestation The curves are in very close agreement with those of Winters, Green and Comstock (1942) for cattle. However the shooting point lies at about 165 days in case of buffalo as against 140 days in cow Curves for other measurements also show almost a similar trend but of different magnitudes as can be seen from graphs 2, 3 4 and 5

The ratios of the body parts to pin-crown length are also quite interesting and have been presented in Table 2 The weight and volume of the specimens in proportion to their length increases steadily throughout the gestation period. The horizontal head circumference, on the other hand decreases proportionately as the specimens grow older The chest and abdomen circumferences are relatively larger in the 45 to 75 days specimens mainly due to the extensive liver prominence at this age. Tail length as well as the length from pin to shoulder point increase uniformly throughout the gestation period except at a few points.

Showing the averages of certain body measurements of the developing buffalo-foetus at successive 30-day periods of gestation.

Sl. No.	Measurements	Stages of gestation in days										
		0—30	31—60	61—90	91—120	121—150	151—180	181—210	211—240	241—270	271—300	At birth
	N *—171	1	39	49	29	20	6	2	2	8	5	10
1	Weight (gm.)	0.06	1.8	14.3	78.0	306.7	1509.1	2440.0	5373.0	9152.0	15955.0	29123.0
2	Volume (cc.)		1.8	13.6	75.5	290.0	1283.0	2375.0	5512.0	9181.0	15862.0	29000.0
3	Pin-crown (cm.)	1.5	2.4	6.1	11.5	19.1	29.5	35.5	48.7	60.0	68.5	74.0
4	Pin-shoulder (cm.)		1.5	3.6	6.6	12.8	18.4	22.5	32.5	41.8	50·0	56.0
5	Tailhead to line joining ears (cm.)		1.9	4.8	9.0	15.1	22.8	30.0	38.6	47.5	52.2	68.0
6	Tail length (cm.)	—	0.8	2.4	4.2	7.1	11.2	16.0	19.0	29.5	34.1	42.0
7	Abdom. circumference (cm.)		3.2	6.5	10.8	17.0	27.4	37.0	45.7	49.2	58.3	72.0
8	Chest circumference (cm.)		2.9	5.8	9.8	15.9	25.8	33.0	43.8	48.2	56.9	70·0
9	Horizontal head circum. (cm.)		2.7	5.5	8.4	13.4	22.0	28.0	32.0	34.5	39.6	42.0
10	Neck circumference (cm.)		1.9	3.6	6.1	9.5	16.0	21.0	25.5	27.0	29.5	36.0
11	Height at withers (cm.)	—	1.3	4.1	7.0	12.2	21.2	28.5	36.5	47.3	53.2	70.0
12	Height at pin (cm.)	—	0.9	3.4	5.2	10.2	16.9	24.0	29.8	40.4	47.0	65.0
13	Chest depth (cm.)		0·9	1.9	3.6	6.1	10.7	15.0	16.5	19.7	21.8	25.0
14	Chest width (cm.)		0.7	1.4	2.6	3.6	6.4	7.8	8.9	11.6	15.0	16.0
15	Head height (cm.)	—	1.0	2.3	4.1	6.5	10.7	19.0	20.6	20.6	20.7	22.6
16	Head length (cm.)		0.9	1.9	3.3	5.0	8.2	10.4	12.1	12.8	14.9	17.5
17	Head width (cm.)		0.8	1.5	2.5	4.1	5.6	8.2	8.8	10·5	11.5	14.0
18	Face length (cm.)		0.4	1.0	1.9	3.1	5.3	9.0	10.0	10.5	12.8	15.0

N = Total number of observations recorded

*This is the contour length and not the Pin-crown length

TABLE 2

Showing the ratios of various body parts to fore-crown length of buffalo foetuses at successive 30-day periods of gestation

S. No.	Stage of gestation in days	N	Weight	Volume	Pin to shoulder	Circumference of Chest	Circumference of Abdomen	Horizontal head	Height at withers
			l_1	l_1	l_1	l_1	l_1	l_1	l_1
1	0—30	1	...					..	..
2	31—60	39	1 3500	1 3800	1 68	0·82	0 76	0 90	1 84
3	61—90	49	0 4100	0 4400	1 69	1 03	0 93	1 11	1 48
4	91—120	29	0 1400	0 1500	1 74	1 17	1 06	1 37	1 64
5	121—150	20	0 0620	0 0630	1 51	1 20	1 12	1 42	1 56
6	151—180	6	0 0220	0 0230	1 59	1 13	1 07	1 53	1 38
7	181—210	2	0 0140	0 0150	1 57	1 11	0 98	1 27	1 25
8	211—240	2	0 0083	0 0084	1 43	1 16	1 12	1 46	1 28
9	241—270	8	0 0063	0 0066	1 43	1 24	1 21	1 74	1 26
10	271—300	5	0 0041	0 0042	1 33	1 25	1 20	1 67	1 23
11	At birth	10	0 0022		1 32	1 03	1 02	1 78	1 05
	Total	171							

N — Number of observations recorded

Discussion

As the material in this study consists of buffalo foetuses of unknown ages, the growth data for cattle have been utilized for determining their ages, assuming the growth rates to be similar in both the cases A relatively large number of specimens, distributed throughout the gestation period have been used for different body measurements and averaged to take care of the individual variation due to age size breeding and care of the pregnant female These values when plotted give much smoother curves than those of Winters, Green and Comstock (1942)

A study of the specimens presented in this text indicates that during the prenatal period the individual undergoes a series of changes in shape, size and disposition The averages of the various measurements clearly indicate that the growth is a continuous process and the various changes merge into one another The growth rate is relatively much faster in the beginning than in the later stages of gestation although the gross development of the foetus is maximum during the later stages only (Fig 25) It is noted that the foetuses gain about 50% of their body weight during the last month, about 25% during the last but one and about 15% during the last but two months of gestation. Thus from the economic point of view it would be needless to emphasise that the dairymen interested in buffaloes should bear the above facts in mind while formulating the feeding schedules and adopting the dairy practices for their pregnant females. This should help them in making due allowance in the feed nutrients for the developing foetuses at different stages of development and provide special care and management to the expectant females and down calvers The curves for weight and volume are in very close agreement with those of Winters, Green and Comstock (1942) for cattle. However from the data presented herein it is apparent that the foetal growth in buffalo is much slower than in Holstein—Friesian, where the birth weight ranges between 70 and 125 lb and is attained in 280 days of gestation. The buffalo can however favourably compare with the light breed of cows like Jersey Making an allowance for the initial difference in the birth-weight and gestation period of buffalo, the findings of the authors are in close agreement (only trend and not the absolute values) with those of Swett et al. (1948) Their averages of certain body measurements of the developing foetuses at successive 30 days intervals for cattle (all breeds combined) and how they compare with our findings can be seen in Table 3

The apparent features of prenatal development in buffalo are very similar to those of cattle and sheep. However there is a considerable difference in their growth rates This may be due to the fact that the buffalo has 1 11 and 2·07 times as long a gestation period as cattle and sheep respectively The rate of prenatal growth in these species is also affected by their average birth weights. The newly born lambs are much

TABLE 3

Showing the comparative average values of certain body measurements in cm. of buffalo and cattle foetuses at successive 30-day periods of gestation.

S No.	Stage of gestation	N Buff in each class	Pin-crown length		Height at withers		Depth of fore-chest		Width of fore-chest	
			Buffalo	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo	Cattle
1	0—30	1	1 5	1 6	..	1 1	—	—		..
2	31—60	39	2 43	4 6	1 32	2 5	0 9	1 9	0 73	1 3
3	61—90	49	6 10	12 0	4 1	6 7	1 9	3 5	1 40	2 2
4	91—120	29	11 52	21 4	7 0	12 1	3 6	5 9	2 60	3 4
5	121—150	20	19 12	33 4	12 2	21 7	6 1	9 6	3 60	3 5
6	151—180	6	29 35	40 1	21 2	27 9	10 7	13 3	6 40	7 2
7	181—210	2	35 50	61 5	28 5	48 0	15 0	17 8	7 80	10 2
8	211—240	2	46 75	72 9	36 5	59 0	16 5	21 6	8 90	11 0
9	241—270	8	60 00	84 3	47 3	68 5	19 7	26 8	11 60	15 8
10	271—300	5	66 50	91 0	53 2	74 8	21 8	..	13 00	—
11	301—330	10	74 00		70 0	..	25 0	..	16 00	—
	N—	171	171	80	170	77	170	45	170	40

This is the curvature length and not the pin-crown length.

N — Total number of observations recorded.

lighter than the newly born calves of cow and buffalo. It is for these reasons that the prefoetal period in buffalo, cow and sheep is 50, and 45 and 34 days and occupies 16%, 16% and 23% of their gestation periods respectively However the stage of foetal development with regard to the size and shape in buffalo, cow and sheep is comparable at 180 140 and 104 days of their respective gestations.

Summary and Conclusion

The shifting interest from cow to buffalo in tropical dairying has been emphasised and the prejudice and paucity of information about buffalo has been pointed out. The importance of information on prenatal development in the efficient calf-raising programmes for profitable dairying has been stressed. The relevant information on this subject has been reviewed and the importance of gathering such an information on buffalo has also been impressed. Observations on the prenatal development of buffalo based on 161 cases of unknown ages, well distributed throughout the gestation period and including both embryos and foetuses irrespective of the sex have been presented, illustrated and discussed.

The ages for the different specimens have been assigned with the help of the data for cows after proper statistical treatment. The specimens under study ranged from 28 days to parturition and were obtained from various sources. The various measurements and observations made on them were after the manner of Winters, Green and Comstock (1942) and Swett *et al.* (1948)

The data collected have been distributed into various age groups of monthly intervals and the class averages for the different measurements have been tabulated and represented graphically The orientational, topographical and morphological changes undergone by the embryos and foetuses during development have also been described and depicted with the help of photographs of the actual specimens. Of the various observations recorded by the authors pin-crown length and weight proved to be more reliable and this reliability was enhanced when both were used together

Some of the general impressions gained during the course of the present investigation are as under —

1 The gestation period of 310 days of this animal can be divided into the following three phases —

(a) Phase of ovum—the period from fertilization to implantation.

(b) Phase of embryo—the period of organo-genesis, and

(c) Phase of foetus—the period from 50 days to parturition.

2. The head undergoes remarkable changes with regard to its contour and disposition. The cephalic dome and cerebral prominence which are maxi

mum during the embryonal stages get progressively reduced during the foetal stage

3 The optic vesicles get established as early as 28 days of intra-uterine life But the actual formation of the eye starts at about the 36th day which gets clearly established by about the 40th day and is covered by the progressively growing eye-lids by the 80th day of gestation.

4 The ear represented by a slit at 36th day stage, gets progressively restricted to a pit and covered by the growing pinna by about the 50th day of gestation

5. The liver prominence on the other hand progressively increases during the embryonal stage, is almost at its maximum by the beginning of the foetal period and then progressively decreases during the foetal phase.

6 Apparently the differentiation of the sexes is not possible until about the 50th day of gestation It is possible only after it enters the foetal period when the genital tubercle starts differentiating into penis of the male or clitoris of the female.

7 The genital swellings which make their appearance irrespective of the sex at about the 45th day of gestation give rise to the scrotum by the 80th day of gestation in males and are lost in females by their incorporation with the vulval lips

8 The mammary glands also develop irrespective of the sex and are apparently visible at the age of about 65 days of gestation. They are disposed differently in the two sexes. They are located on an arc in males and almost squarely placed in the females.

9 The black pigmentation of skin characteristic of buffalo, starts from about the 150th day and is almost completed by about the 200th day of gestation

10 Hairs grow much later than the start of pigmentation (175 days stage) and the coat cover is complete only by about the 275th day of gestation.

11 Limbs which represent the degree of extreme specialization make their appearance as limb buds as early as in 32nd day specimen These grow progressively as simple outgrowths and then get differentiated with hooves dew claws and various joints and points by about the 50th day of gestation Their further elaboration is brought about during the foetal phase

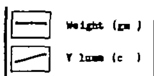
12 The apparent rate of development is very slow in the beginning (only about 10% of the birth weight during the first 7 months) and extremely rapid during the last 3 months of gestation (about 90% of the birth weight)

Thus on the basis of this investigation, it can safely be recommended that the last three months of gestation is the most critical period for any progressive dairy enterpriser dealing in buffaloes because 90% of the total foetal growth takes place during this part of the gestation. The feeding schedules and the management practices for the pregnant females should therefore be adjusted accordingly for successful dairying.

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Graph 1 : Showing the rate f gain in foetal weight and volume during prenatal devel pme t



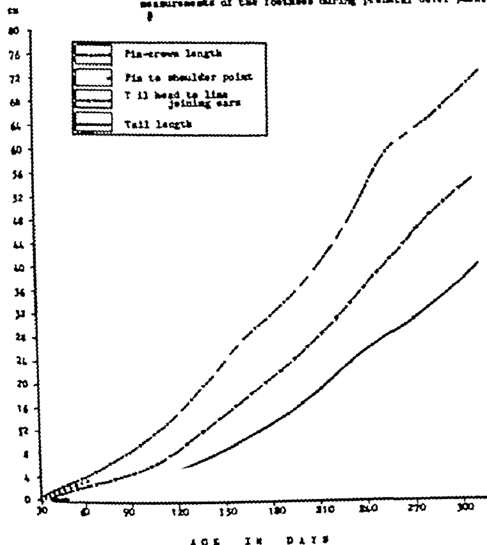
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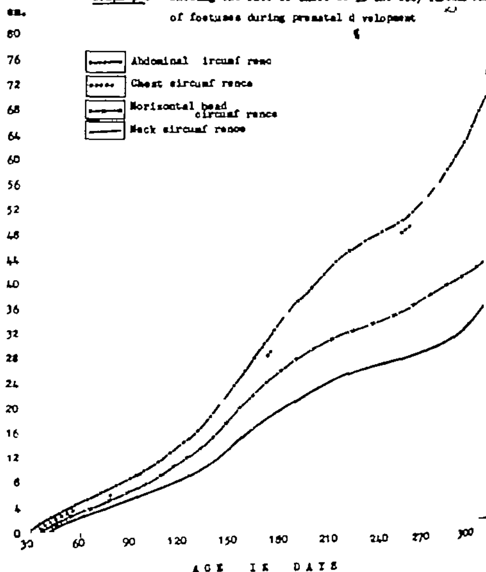
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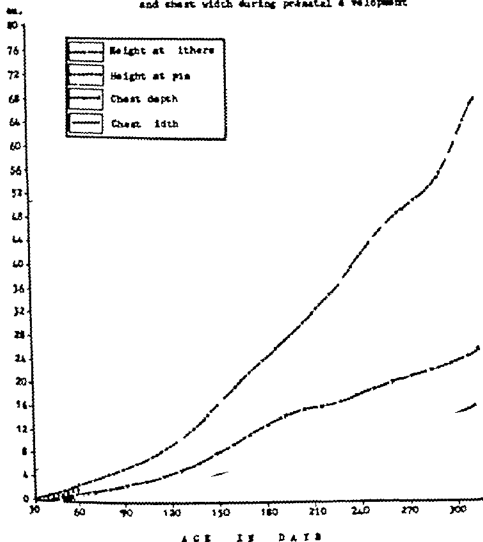
AGE IN DAYS

Graph 2 - Showing the rate of increase in the linear body measurements of the foetuses during prenatal devel pment





Graph 3 : Showing the rate of incre se in the body circumf rence of foetuses during prenatal d velopment



Graph 4.1 Showing the rate f increase in the foetal height and chest width during prenatal d velopment

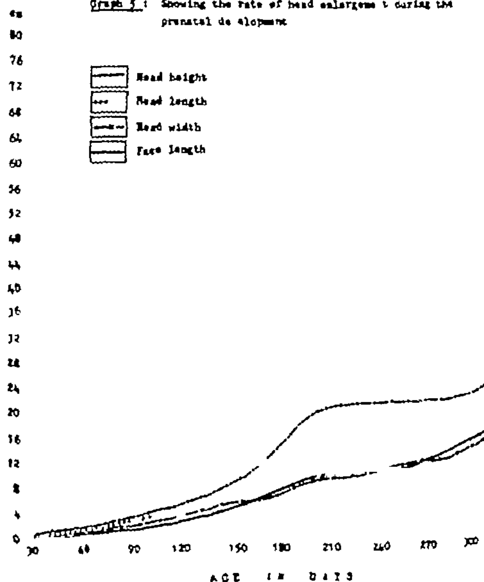
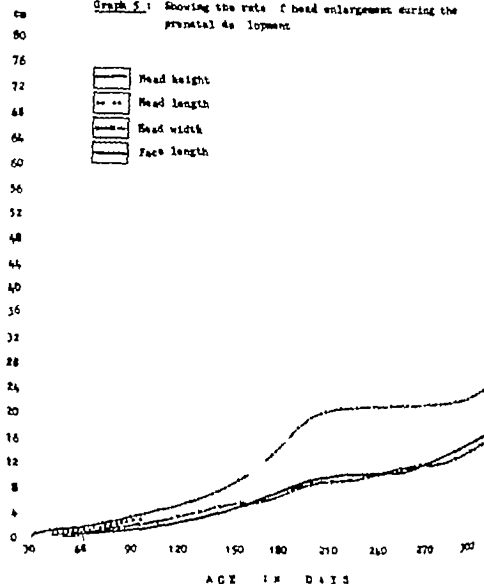

Graph 5 : Showing the rate of head enlargement during the prenatal development
Head height
Head length
Head width
Face length
84
80
76
72
68
64
60
56
52
48
44
40
36
32
28
24
20
16
12
8
4
0
30
60
90
120
150
180
210
240
270
300
AGE IN DAYS



Fig. 1—An intact gravid uterus with pregnancy in the right horn



Fig 2—Gravid uterus opened to show the chorion and ts attachment with terior wall as cotyledons.



Graph 5 : Showing the rate of head enlargement during the prenatal development

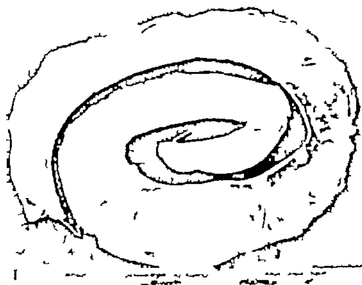


Fig 3—Foetus inside the Amnion and chorion after it is taken out of the uterus.



Fig 4—Horn opened under magnifying lens for th collection of the early embryos

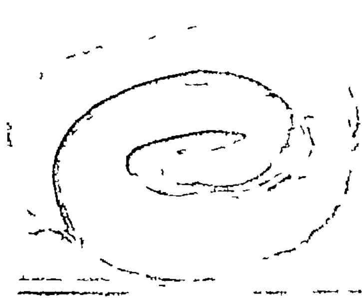


Fig. 3—Foetus inside the Amnion and chorion after it is tak... f the ... terus.

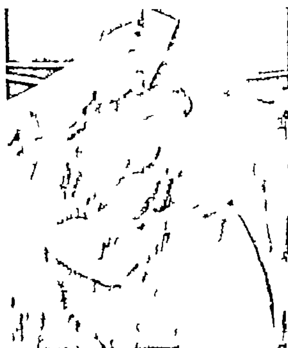


Fig. 4—Horn opened under magnifying lens for the collection of the early embryos.

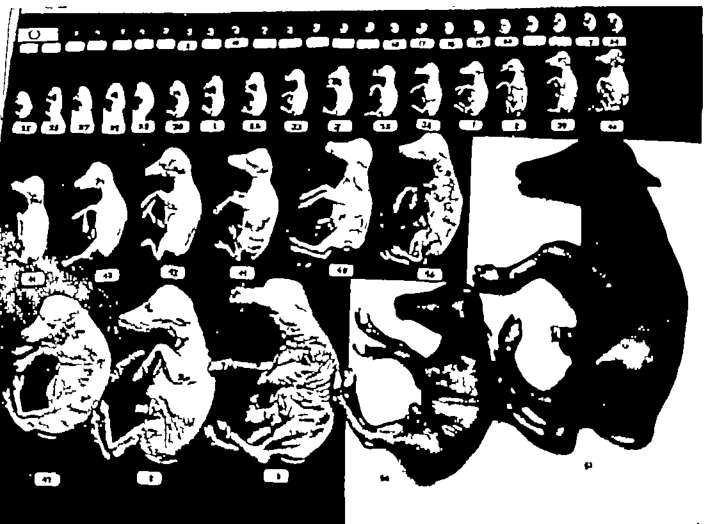
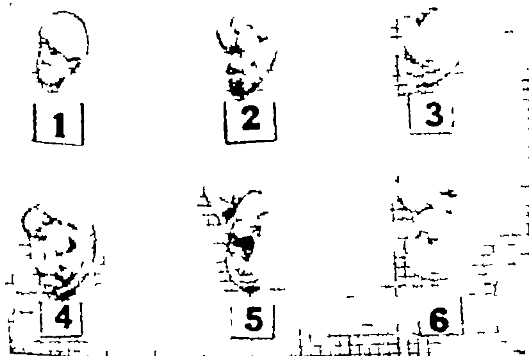


Fig. 3—Embryos and foetuses from 28 day (top extreme left on slide) to 210 day (bottom extreme right) gestation arranged serially



les of early embryos showing the progressive changes i their contour characteristics and rganogeny



Fig 7

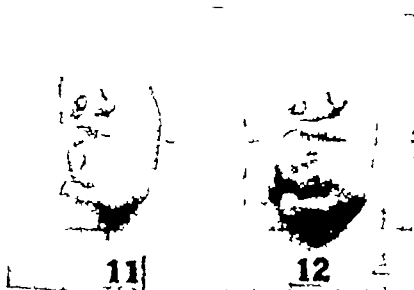


Fig 8

Figs 7 & 8—A series of advanced embryos showing the progressive changes in their contour characteristics and organogeny



Fig. 9—A 28 day embryo with characteristic 'C' shaped structure prominent cephalic heart liver and mesonephric prominences optic and otocyst vesicles and the branchial arches.

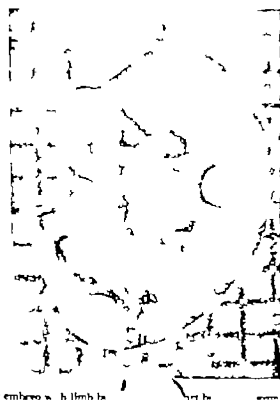


Fig. 10—A 32 day embryo with limb b[illegible] art b[illegible] liver prominence



Fig 11—A 36 day embryo with reduced branchial arches clearly defined eye spot and ear slit and enlarged liver prominence

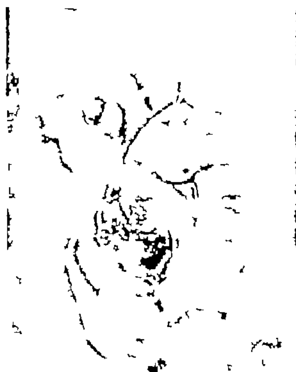


Fig 12—A 40 day embryo with marked mouth and characteristic li extended contour of bod



Fig 13—A 45 day embryo with prominent cephalic dome sinking eye, pinnaless ear pit, and snouted face.



Fig 14—A 50 day embryo with receding cephalic dome & growing cerebral bulge, a large lever prominence growing eye-lids and pinna and elaborated limbs with regard to their joints points and bones



Fig 15—A 63 day foetus with partially covered eyes by the growing eye-lids.



Fig. 16—A 80 day foetus with meeting eye-lids and the head increasing in its angle with the main axis.

17

Fig 17—A 100 day foetus with fused eye lids over the eyes and head oriented at 90° with the main axis of the body

18

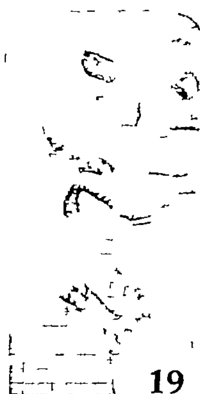


Fig. 19—A 150 day foetus with black skin pigmentation just started



Fig. 20—A 175 day foetus with hairs on muzzle eyelids



Fig. 21—A 203 day foetus with completely pigmented black skin and hairs on eye-brows also.



Fig. 22—A 240 day foetus with horn spots and hairs starting to grow over the body

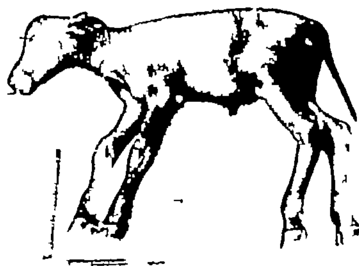


Fig. 23—A 290 day foetus with horn buttons and sparsely hair coated

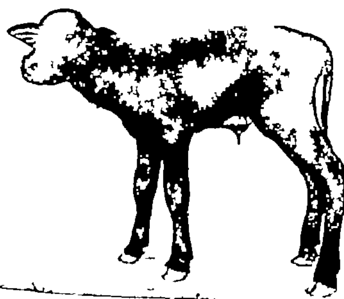


Fig. 24—A day old calf normally born after gestation period of 309 days.

THE STRUCTURE AND DEVELOPMENT OF SEEDS IN CONVOLVULACEAE IPOMOEA SPECIES

J S Jos

Department of Botany B R. College Agra.

The genus *Ipomoea* belongs to the family Convolvulaceae which is repre sented in India by 15 genera and 146 species (Hooker 1883) The genus *Ipomoea* is of much interest because of its economical and horticultural importance. In the family the genus *Cuscuta* has received comparatively more attention from earlier times from various workers because of its parasitic nature and undiffer entiated embryo (Peters 1908 Fedortschuk, 1931 Johri and Nand 1934 Smith 1934 Finn, 1937 Tiagi 1951 Johri and Tiagi 1952) Though diffe rent workers (Kenyan, 1929 Juliano 1935 Tiwari, 1936 Rao 1940 Woodcock 1942 Sripleng and Smith 1960) have contributed to the knowledge of embryo-geny of the different genera belonging to Convolvulaceae the literature on the seed structure of the genus *Ipomoea* is rather scanty

The present work has been undertaken at the suggestion of Dr R. P Singh, Associate Professor of Botany B. R. College, Agra to evaluate and com pare the seed structure in two species of *Ipomoea* namely *I pes-tigridis* Linn. and *I. aquatica* Forsk with the seed structure of other genera of the same family particularly on the basis of seed coat anatomy The former species dominates the waste places in the rainy season while the latter is a hydrophyte The present study also includes a reinvestigation of the embryogeny of *I pes-tigridis*.

Materials and Methods

The flowers were collected locally early in the morning and late in the evening The sepals, petals and stamens were removed before fixing in formalin-acetic-alcohol The mature ovules were dissected out and an incision was made on the side to facilitate easy infiltration. Some difficulty was experi enced during infiltration due to the thick walled palisade sclerenchymatous layers in the mature ovules The material was run through ethyl alcohol xylol series and embedded in paraffin. Transverse and longitudinal sections of ovules and young buds were cut 8-15 microns thick. To get the complete picture of the seed coat anatomy sections in oblique planes were also obtained The sections were stained in Heidenhains iron haematoxylin and counter stained with fast green or safranin fast green combination was used and the latter turned to be more satisfactory for this study Mature embryo sacs and young embryos were dissected out under the binocular Jeffrey's maceration technique was also employed to study the seed coat structure of mature seeds.

Observations

Ovule

Flowers of both the species (*I pes-tigridis* and *I aquatica*) have pentamerous flowers (Fig 1) with a 3 or 4 loculed gynoecium having 1 or 2 ovules in each loculus, a globose ovary and a long style with two lobed papillose stigma. The ovules are more or less basal and develop from an axile placenta. The nucellar primordia arise as protruberances on the placenta and are erect in the beginning but soon become inverted (Fig 2) They are tenuinucellate and unitegmic. The integumentary primordium is initiated at the bottom of the ovule and later it grows beyond the nucellus forming a distinct micropyle. Woodcock (1942) failed to distinguish between the integument and nucellus in *I rubro-caerulea* and considered that the micropyle is formed owing to an invagination of the ovule next to the funiculus This observation, however was refuted by Maheshwari (1944) in view of reports of Kenyan (1929), Juliano (1935) and Rao (1940) In both the species studied by the present author the micropyle is quite distinct and the nucellus disappears at an early stage

There is a short funicle through which the vascular trace enters and travels up the funicle, reaches the chalaza and descends on the other side of the ovule to terminate near the micropylar end of the ovule (Fig 3) Integumentary vascular bundles have been reported in several families of angiosperms (Maheshwari 1950) but the extension of vascular trace beyond the chalaza to reach almost the micropylar tip as in this case seems to be noteworthy Smith (1934) reported a similar extension of tracheids beyond the chalaza in the young ovules of *Cuscuta arvensis* and considered it as a very unusual feature for angiosperm.

The integument shows an epidermis made up of rectangular cells with prominent nuclei A single layer of hypodermal cells are present beneath the epidermis The hypodermis is followed by about 15 layers of parenchymatous cells and they divide quite often and at the mature embryo sac stage there are about 25 to 30 layers of parenchymatous cells They grade in size from small cells near the periphery to larger cells towards the embryo sac (Fig 4). The parenchymatous cells contain starch grains and they are absent in the epidermis, hypodermis and the cells bordering the embryo sac. Mac Pherson (1921) stated that the cells of the nucellus are rich in starch but Dubigres (1927) pointed out that these cells may belong to the integument. From the present study it is quite evident that the starch grains are present in the cells of the integument and the nucellus is ephemeral. The cells which border the mature embryo sac are in a demolished condition. The structure of the integument is quite uniform and no difference is noted between the sides and the region in between the hilum and the micropyle

Megasporogenesis and female gametophyte

The megaspore mother cell is present directly beneath the epidermis. It divides meiotically to form a linear tetrad of megaspores in which the chalazal one functions and the other 3 degenerate. Rao (1940) has reported the presence of parietal cells in *I. learii*, while Peters (1908) has reported the same in the nucellus of the ovules of *Convolvulus arvensis* and *Cuscuta*, but it was questioned by Dahlgren (1927). Mathur (1934) confirmed the observation of Peters (1908) on *Convolvulus arvensis*. Parietal cells are not observed in the present study.

The female gametophyte is 8 nucleate. The antipodals are ephemeral and disappear at an early stage (Fig. 5). In Convolvulaceae the female gametophyte is monosporic 8 nucleate (Rao, K. V. R. 1940, Rao, V. S. 1944, Tiagi 1951, Sripleng and Smith, 1960) with the only exception of *Cuscuta reflexa* where the female gametophyte is reported to be bisporic 8 nucleate (Johri and Nand, 1934, Johri and Tiagi, 1952).

The mature embryo sac is narrow, erect and extends almost throughout the length of the ovule, but at globular embryo stage the embryo sac is deeply two lobed at the bottom and narrower at the micropylar end (Fig. 6). Juliano (1927) observed long, narrow and curved embryo sac in *I. trifida*, *Cuscuta Gronovii* and *Convolvulus sepium* and considered that this type is typical to Convolvulaceae. Later workers did not mention any such type and in the present study also the embryo sac is found to be erect. Therefore, his conclusion seems to be doubtful.

In *I. pes-tigridis* the two synergids are somewhat pointed towards the micropylar end. Hook synergids are reported in 8 other species of *Ipomoea*, *Argyreia speciosa* and *Evolvulus alsinoides* by Rao (1940), *I. pulchella*, *Operculina turpethum* and *Jacquemontia violacea* by Rao (1944) and in two species of *Cuscuta* by Tiagi (1951). In *I. aquatica* the egg and the two synergids are highly inflated and vacuolated (Fig. 7). In both the species the polar nuclei lie side by side and they do not fuse till the entrance of the pollen tube.

The cells of the placenta just around the funicle on the innerside begin to divide and fill up the space between the carpellary wall and the micropylar region of the ovule. The micropyle is very closely appressed with the obturator. The obturator is not observed in the young ovules and develops only at a later stage (Fig. 8).

Pollination and fertilization

One of the synergids is destroyed by the entrance of the pollen tube but the second synergid persists for a longer period. In an ovule of *I. pes-tigridis* two pollen tubes are noted and one of them shows two nuclei. They may be the male gametes of the second pollen tube. The actual fusion of the egg nuc

leus with the male gamete is not observed in the present study The two polar nuclei and the second male gamete fuse almost simultaneously

Endosperm

The division of the primary endosperm nucleus and that of the zygote occurs almost at the same time Since the earlier divisions are not accompanied by wall formation the endosperm is free nuclear Later the wall formation is initiated at the micropylar end from where it proceeds towards the chalaza.

Embryo

The first division of the oospore is transverse (Fig 9) to form a terminal cell (ca) towards the chalazal end and a basal cell (cb) towards the micropylar end The two cells are quite unequal and the basal cell is much more bigger than the terminal cell The basal cell may divide immediately by a longitudinal wall (Fig 11) or it may divide only after the terminal cell has undergone a few divisions (Fig 16) The embryo proper is derived from the terminal cell. The terminal cell divides transversely to form l towards the basal cell and l towards the chalaza The cell l which has a terminal position divides by a transverse wall while the cell l which has a subterminal position divides only longitudinally This sequence of transverse and longitudinal divisions of the cells having terminal and subterminal position is long maintained in the development of the embryo (Figs. 10-19) The pattern of embryogeny observed in the present study closely resembles that observed by Rao (1940)

Some variation in the development of the proembryo is also observed in *I pes-tigridis* In one case a 3-celled proembryo shows that the basal cell and the other two cells formed from the terminal cell are alike in size (Fig 20) In three instances 6-celled embryo shows a linear arrangement of their cells (Figs 21 23) This type of linear embryo might have arisen from the type of embryo shown in Fig 20 in which the basal cell and the other two cells derived from the terminal cell look alike. In later stages linear embryos are not seen Such a linear 6-celled proembryo, by longitudinal divisions in the cells derived from the terminal cell, and radial and longitudinal divisions in the cells derived from the basal cell can give rise to a condition similar to that shown in Fig 17

The basal cell forms a very massive suspensor The suspensor is somewhat globular in *I pes-tigridis* while almost pyramidal in shape in *I aquatica* Rao (1940) has also reported that the suspensor in *I. hederacea* is pyramidal in shape while globular in *I hederacea* In *I aquatica* the suspensor is more massive than in *I pes-tigridis*

In later stages each cotyledon is two lobed and the cells of the cotyledons are not homogeneous. Among ordinary compact parenchymatous cells other cells having enormously big size with prominent nuclei are present. The cells contain numerous starch granules except that large cells. In *I pes-tigridis* the cells of the mature folded cotyledons contain calcium oxalate crystals and the folding of the cotyledons may be due to the lack of available space.

Seed Coat

Before fertilisation the integument is composed of uniform cell but soon after fertilisation rapid changes occur in the micropylar region. In this region the cells of the epidermis and hypodermis begin to divide and the number of cell layers is thereby increased The epidermis divides to form about 4 or 5 layers of parenchymatous cells and the hypodermis gives rise to two layers of cells which become radially elongated to form the palisade layer of the seed coat. The cells of the outer palisade layer divide only once so that there are only two layers of palisade cells towards outside while the cells of the inner palisade layer undergo more divisions, so that there are 3 cells in each row Finally the cells of both the palisade layers become thick walled and sclerenchymatous (Figs. 25-27)

In other regions after fertilisation only the subhypodermal cells divide to increase the thickness of the integument It is only after the initiation of the cotyledonary primordia that the cells of the hypodermis undergo periclinal divisions to form two layers. The cells of the outer layer do not show any conspicuous change while the cells of the inner layer undergo radial elongation to form the palisade layer of the seed coat. Each palisade cell may divide to form 2 or 3 radially elongated cells (Figs. 28-32) The palisade cells become thickwalled and sclerenchymatous and are in direct continuation with the palisade sclerenchyma of the micropylar region Some epidermal cells develop into unicellular elongated hairs. At later stages the parenchymatous cells are reduced to about 10 layers due to the increase in size of the embryo sac.

The cells inner to palisade layer are thinwalled parenchymatous cells and contain starch grains in the outer layers only In the fully mature seed only 2 to 3 layers of parenchymatous cells are left. Later the epidermis and hypodermal layer may peel off leaving the palisade sclerenchyma as the outer most layer of the seed coat.

Woodcock (1942) described briefly the seed coat of *I rubrocaerulea* He has reported a single layer of palisade sclerenchyma and the presence of blunt, one-celled epidermal hairs on the surface and thick-walled epidermis. While Juliano (1935) found two layers of palisade sclerenchyma in which the outer is more elongated than the inner in *I batatas* Two layers of palisade sclerenchyma are reported by Tigal (1951) in *Cuscuta hyalina* and *C planiflora* and

by Johri and Tiagi (1952) in *C reflexa*. In *Convolvulus arvensis* the work of Sripleng and Smith (1960) revealed that the palisade sclerenchyma on sides may be 1 2 or 3 layers depending upon the sequence of anticlinal and periclinal divisions. Both the species under the present investigation show 2 or 3 palisade sclerenchymatous layers on the sides and about 5 layers in the micropylar region In the micropylar region of *I pes-tigridis* and *I aquatica* between the epidermis and the palisade sclerenchyma there are 4 layers of parenchymatous cells. Sripleng and Smith (1960) celled these layers as multiple epidermis while describing the seed coat of *Convolvulus arvensis*. In *Cuscuta* and the epidermal cells are thin walled and contain starch. In *I pes tigridis* and *I aquatica* also the epidermal cells are thin walled and starch is absent and the epidermal hairs one-celled and pointed From seed coat anatomy it is quite evident that almost all the different genera of Convolvulaceae possess strikingly the same structure and the seed coat anatomy should be considered as a family character rather than that of a genus or a species character

Abnormalities

In one of the dissected ovules two embryo sacs are noted in an ovule of *I pes-tigridis* A similar observation was made again in serial sections in which each of the two embryo sacs had two synergids an egg and two polar nuclei

A 3-celled proembryo is observed inside an unopened bud of *I pes-tigridis* and in the micropyle of this ovule a pollen tube is also present. The presence of pollen tube in the micropyle indicates that anthesis has already taken place in the bud condition and self pollination was effected Since the plant flowers in the rainy season the self pollination in the closed buds may be an adaptation.

Two fully developed embryos along with an aborted 3-celled embryo was found in an ovule of *I pes-tigridis* (Fig 24)

DISCUSSION

The present study has revealed several features of interest like the occurrence of twin embryo sacs two types of embryo development polyembryony and cleistogamy

The occurrence of the second embryo sac in the same ovule is of much interest in view of the occurrence of polyembryony in certain species of Convolvulaceae In *I pes-tigridis* two ovules are noted with two embryo sacs in each. In the present study serial sections clearly show two synergids, an egg and two polar nuclei in each embryo sac. In *Cuscuta reflexa*, Johri and Tiagi (1952) considered that if two megaspore mother cells function simultaneously in the same nucellus, they can give rise to two embryo sacs

and in case the wall disappears all the nuclei come together and organise in a different way

In one well developed ovule of *I pes-tigridis* two fully developed embryos with an aborted 3-celled embryo are noted. Macpherson (1921) reported numerous instances of polyembryony in *Convolvulus sepium*. In *Cuscuta reflexa* polyembryony is observed sometimes, the additional embryo being derived from one of the synergids (Johri and Tiagi 1952) But Sripleng and Smith (1960) deny the occurrence of polyembryony in *Convolvulus arvensis* In *I pes-tigridis* the two fully developed embryo might have developed from the two eggs of two different embryo sacs and the wall disappears between the two embryo sacs, so that in later stages it appears as though both embryos occur in the same embryo sac. The origin of the aborted 3 celled embryo is doubtful but the position indicates that it might have arisen from one of the synergids

In the present study on *I pes-tigridis* two distinct types of embryo development seem to take place One type belongs to the pattern described by Rao (1940) Regarding the second type 3 proembryos are found which differ radically from the first type. The proembryos are 6-celled and the cells are linearly arranged and later it seems that they develop into normal embryos. Two types of embryo development are also described for *Cuscuta* (Fedortschuk, 1931 Tiagi 1951 Johri and Tiagi, 1952) but they differ only in the nature of their suspensor cells. Rao (1940) observed a 7-celled filamentous embryo in *Evolvulus alsinoides* Tiwari (1936) cited a 6-celled suspensor in *E. nummularius* and from the end cell of which the embryo is described to have organised

Johansen (1950) includes *I hederacea*, *I learii* and *Argyreia speciosa* in Caryophyllad type Myriophyllum variation where the basal cell divides to form two juxtaposed inflated cells But in *I pes-tigridis* and *I aquatica* t is quite evident that the basal cell by repeated divisions forms a massive suspensor So it seems logical to include these two species in Caryophyllad type, Fumaria variation as described by Johansen (1950)

SUMMARY

The pentamerous flower has a gynoecium of 3 or 4 chambers and each loculus shows one o two ovules attached on the axile placenta

The unitegmic, tenuinucellat ovule is anatropous. The placenta proliferates at the base of each ovule to form an obturator which lies in close proximity with the micropyle. The vascular supply to the ovule extends nearly upto the tip of the integument

In young ovules the embryo sac is narrow and elongated but in later stages it becomes two lobed towards the chalazal side. In *I pes-tigridis* some times two embryo sacs are present.

Double fertilisation occurs. The fusion of the egg nucleus with the male gamete occurs almost simultaneously with the fusion of the polar nuclei and the second male gamete.

Synergids are pointed towards the micropylar end. In *I. aquatica* the synergids and the egg are extremely large in size and much vacuolated. At the entrance of pollen tube one synergid is destroyed but the other persists for sometime.

The zygote and the primary endosperm nucleus divide at about the same. The endosperm is free nuclear but later becomes cellular and the walls are laid down at the micropylar end first and proceeds to the chalazal end.

First division of zygote is transverse. The basal cell forms the massive suspensor while the terminal cell organises the embryo proper. The embryogeny follows that of Caryophyllad type Fumaria variation. In *I. pes-tigridis* linearly arranged 6-celled proembryo is noted in three cases. In one instance a 3-celled embryo is noted inside a bud where the petals have not opened and a pollen tube is present in the micropyle. In the same species polyembryony also occurs.

The cotyledons are folded and certain cells contain calcium oxalate crystals.

The seed coat consists of epidermis and hypodermis and two or three layers of thick walled palisade sclerenchymatous cells, but in the micropylar region 5 layers of radially stretched cells are present. There are only about 10 layers of parenchymatous cells and others are consumed by the embryo.

ACKNOWLEDGEMENTS

I am deeply indebted to Dr R. P Singh for suggesting this problem and guiding throughout this study. I also wish to thank Prof S N Chaturvedi and Dr S P Singh for all the help that they extended during this study and also to Dr R. K. Singh, Principal, B R. College Agra for providing the necessary facilities for the research work.

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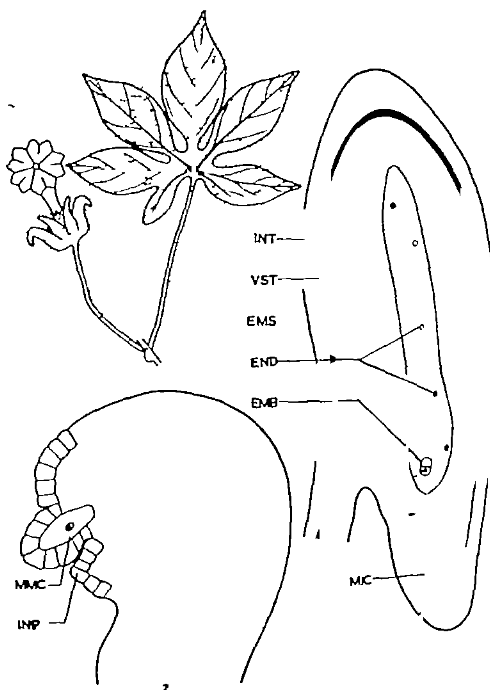


PLATE I

Figs. 1-3. Twig and ovule of *I. pes-tigridis* (EMB, embryo; EMS, embryo sac; END, nuclear endosperm; INP, integumentary primordium; INT integument; MMC, megaspore mother cell; VST vascular trace).

Fig 1 Twig (natural size)

Fig 2. Young anatropous ovule. × 490

Fig 3. Vascular supply of the ovule. × 210.

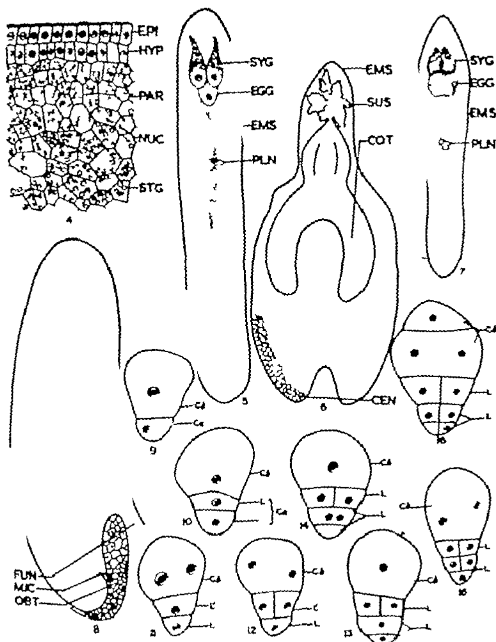


PLATE 2

Figs. 4-6 and 8-16. I. pes-tigridis. Fig. 7. I. aquatica (Ca, terminal cell; Cb, basal cell; CEN, cellular endosperm; COT, cotyledon; EMS, embryo sac; EPI, epidermis; FUN, funicle; HYP, hypodermis; MIC, micropyle; NUC, nucleus; OBT, obturator; PAR, parenchymatous cells; PLN, polar nuclei; STG, starch grains; SUS, suspensor; SYG, synergids)

Fig. 4. L. S. of integument of young ovule. X 420.
Fig. 5. Embryo sac. X 420.
Fig. 6. Two lobed embryo sac. X 420.
Fig. 7. Embryo sac showing vacuolated synergids and egg. X 125.
Fig. 8. Ovule with obturator. X 125.
Fig. 9. Embryogeny. X 533.

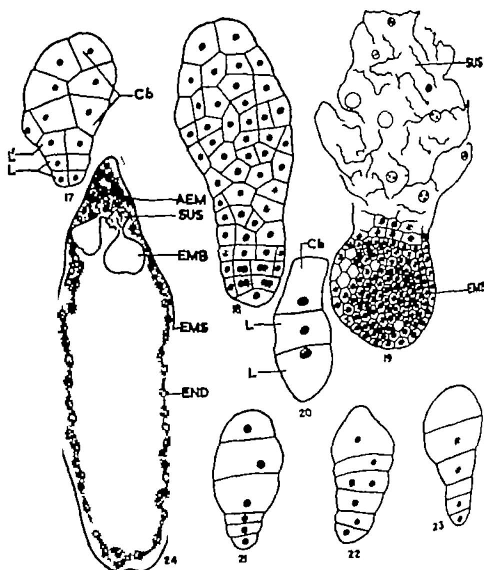


PLATE 3

Figs 1 24 *L. pen-digitalis* (AEM aborted embryo; Cb, basal cell, EMB, embryo; EMS embryo sac; END nuclear endosperm; SUS suspensor).

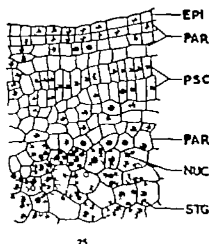
Figs 1 18. Embryogeny X 525.

Fig. 19 Globular embryo with suspensor X 40.

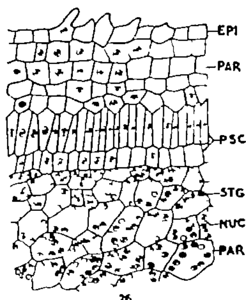
Fig 20. 3-celled embryo where all the cells are alike in size. X 525.

Figs. 21 23. Linear 6-celled embryos X 525.

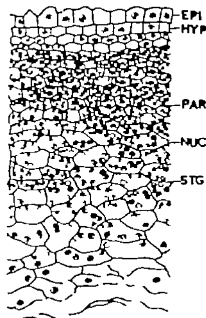
Fig 24 Polyembryony X 120



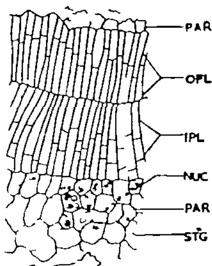
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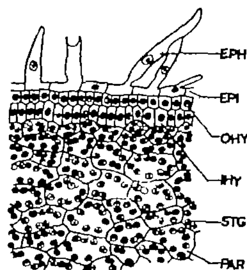
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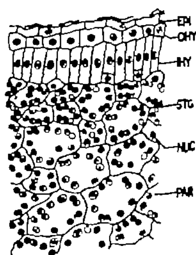
PLATE 4

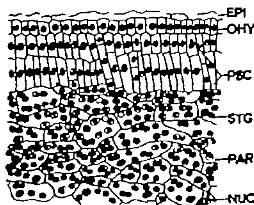
Figs. 25-28 *I. pes-tigridis* (EPI, epidermis; IPL, inner palisade sclerenchyma; NUC, nucleus; OPL, outer palisade sclerenchyma; PAR, parenchymatous cells; PSC, palisade sclerenchyma; STG, starch grains)

Figs. 25-27 L. S. of seed showing seed coat development at the micropylar region. Figs. 25 and 26 X 420 Fig. 27 X 125.

Fig. 28. L. S. of young seed showing seed coat structure at the sides. X 420.







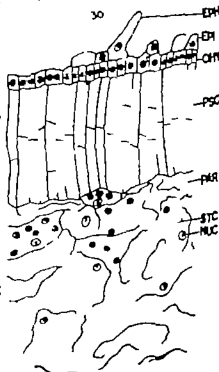


PLATE 5

Figs. 29-32. *I. pes-tigridis* (EPH, epidermal hair; EPI, epidermis; IHY, inner hypodermis; NUC, nucleus; OHY, outer hypodermis; PAR, parenchymatous cells; PSC, pallisade sclerenchyma; STG, starch grains)

Figs. 29-32. T. S. of older seed coat development at the sides × 420.

EFFECT OF SOIL TEMPERATURE ON ROOT ROT OF GUAR (*CYAMOPSIS PSORALIOIDES* DC.) AND WILT OF GRAM (*CICER ARIETINUM* L.) CAUSED BY *SCLEROTIUM ROLFSII* SACC.[1]

S. B. MATHUR*

Botany Department, Agra College, Agra

INTRODUCTION

Pioneer work on the effect of soil temperature on disease development was conducted by Jones and his collaborators (1926) at the University of Wisconsin. The Wisconsin School developed the well known soil temperature tanks for study under controlled temperatures. Such experiments were supplemented by the determination of cardinal temperatures for the growth of the parasite in pure culture, which were often found closely corresponding with temperatures favouring optimum disease development. On the basis of the reaction to soil temperature, Garrett (1944) grouped the soil borne diseases into categories as favoured by (a) low temperature or (b) high temperature.

This paper deals with the study on the influence of temperature on the development of root-rot of guar (*Cyamopsis psoralioides* DC.) and wilt of gram (*Cicer arietinum* L.) and also on the vegetative growth of *Sclerotium rolfsii* Sacc. which is the causal organism.

METHOD AND MATERIAL

The influence of seven temperatures viz. 15°, 20°, 25°, 30°, 34°, 39° and 42°C on the linear spread of *Sclerotium rolfsii* cultured in standard Czapek's medium (Rawlins 1933) was determined. Five replications were maintained for each treatment. Growth was measured as mean of two diameters of a colony. The data on total growth on the seventh day was subjected to statistical analysis, critical difference (C. D.) being calculated following the analysis of variance method.

Glazed aluminium tumblers (10″ × 5″) filled with acid washed Chambal[2] sand were used to raise the seedlings and were fitted in soil temperature tanks (±2°C) designed after those of Wisconsin. The various temperatures maintained were 20°, 25°, 30°, 34°, 39° and 42° C. The pots were irrigated with equal amounts of modified Shive three salt nutrient solution ($CaNO_3$-2·22 gms, $MgSO_4$-1·12 gms and KH_2PO_4-0·66 gms in one litre of tap water) at suitable intervals. The inoculum of the pathogen was raised on autoclaved corn meal sand medium and equal quantities of the

[1] A part of the Thesis approved for the Ph. D. degree of Agra University, Agra.

[2] Name of river in India which deposits sand which is neutral.

* Present address—Research Associate in Plant Pathology, Macdonald College of McGill University, Dept. of Entomology & Plant Pathology, P. Q., Canada.

same were mixed thoroughly with the sand of the experimental pots seven days before sowing Five seeds were sown in each pot. For each treatment, 5 inoculated and 1 uninoculated (as control) pots were kept. Observations on pre-emergence mortality (includes rotting of seeds and infection of young seedlings before emergence) and post-emergence seedling mortality for a period of 44 days were taken at an interval of 4 days. In control pots, the seedlings remained healthy throughout the period of investigation in all the treatments under observation Critical differences were calculated for total percentage mortality (pre and post-emergence mortality together)

RESULTS

TABLE 1

Showing total vegetative growth of S ROLFSII on Czapek's Medium. Average colony diameter in millimeters

(Mean of 5 replications)

Temperature in Degree Centigrade							
15	20	25	30	34	38	42	C. D at 5% level
31 8	46·0	87·0	105 3	59.8	26·2	10·2	16·13

The above table indicates that the parasite can grow at all the temperatures under study The linear growth increases with rise in temperature from 15°C onward and is maximum at 30° C but further increase in temperature reduces the same. In the order of their effectiveness favouring growth the various temperatures can be arranged as 30°C (105 3 mms) < 25°C (87·0 mms) < 34°C (59·8 mms) < 20°C (46·0 mms) < 15°C (31·8 mms) < 38°C (26·2 mms) < 42°C (10·2 mms)

The data included in Table 1 show that there are six groupings of temperatures on the basis of critical difference.

42°C	38°C	15°C	20°C	34°C	25°C	30°C
10·2	26·2	31 8	46.0	59·8	87·0	105·3

C. D 16 13

The above grouping clearly indicates the independent behaviour of the fungus at 25° and 30°C while other observed temperatures have a tendency for intermixing as shown above

SOIL TEMPERATURE

ROOT ROT OF GUAR

TABLE 2

Showing percentage emergence of seedlings and percentage pre-emergence mortality in guar

Temperature in °C	Emergence of seedlings: Control series	Emergence of seedlings: Infested series	Pre-emergence mortality
20	100	88	12
25	100	76	24
30	100	80	20
34	100	96	4
38	100	96	4
42	100	100	0

The lowering in emergence of seedlings in the infested pots was found to be due to the attack of the parasite which was isolated invariably from all those seeds that did not germinate. The parasitic activity of the parasite was most severe at 25°C and 30°C, the actual mortality figures being 24 and 20% respectively. It decreased considerably at high temperatures.

TABLE 3

Showing progressive post-emergence percentage seedling mortality in Guar at various temperatures

Days after sowing	Temperature in Degree Centigrade: 20	25	30	34	38	42
4	0·0	0 0	0 0	0 0	0 0	0·0
8	0·0	0 0	0 0	4 0	0 0	4·0
12	0·0	0 0	0 0	8 0	0 0	4·0

(Continued on page 264)

TABLE 3 (*Contd.*)

Days after sowing	Temperature in Degree Centigrade					
	20	25	30	34	38	42
16	0·0	0 0	4 0	16 0	4 0	40
20	8·0	8 0	28 0	20 0	8 0	40
24	12·0	16 0	32 0	28 0	12 0	40
28	12 0	20 0	40 0	32 0	16 0	40
32	12·0	20 0	48 0	32 0	16 0	40
36	12·0	24·0	48 0	32 0	16 0	40
40	12·0	32 0	52 0	32 0	16 0	40
44	12·0	32 0	52 0	32 0	16 0	40

Table 3 reveals that the total post-emergence mortality increase progressively with rise in temperature from 20° C to 30° C where a maximum value of 52% is reached but further increase in temperature lowers the same. It is interesting to note that the seedling mortality (32%) was the same at 25° C and 34° C.

TABLE 4

Showing total percentage mortality in Guar

(Average of 5 pots, each with 5 seeds)

Temperature in Degree Centigrade						C. D. at 5% Level
20	25	30	34	38	42	
24 0	56 0	72 0	36 0	20 0	4 0	18·3

The above table indicates that the total percentage mortality is maximum at 30° C and it decreases with increase or decrease in temperature more so in the higher ranges Further the difference in mortality figures between 20° 34° and 38° C 38° and 42° C and 25° and 30° C are

statistically non-significant (because the differences in between them are less than the C. D which is 18 5) The six temperatures employed are grouped below

42° C	38° C	20° C	34° C	25° C	30° C
4·0	20·0	24·0	36·0	56·0	72·0

C. D is 18·5

The above grouping of temperatures, on statistical basis, clearly indicates that the temperature range of 25°-30° C favours disease development the most.

WILT OF GRAM

TABLE 5

Showing percentage emergence of seedlings and also percentage pre-emergence of mortality in gram.

Temperature in °C	Emergence of seedlings		Pre-emergence mortality
	Control series	Infested series	
20	100	92	8
25	100	96	4
30	100	76	24
34	100	100	0
38	100	100	0

Note—Temperature 42°C was not taken as germination of gram was not normal in the control pots.

It is evident from Table 5 that pre-emergence mortality in the infested pots was 8, 4 and 24% at 20°C, 25° C and 30° C respectively Thus parasitic

activity of the fungus was most severe at 30°C and no pre-emergence infection was noted at 34° and 38° C.

TABLE 6

Showing progressive post-emergence seedling mortality in Gram.

Days after sowing	Temperature in Degree Centigrade				
	20	25	30	34	38
4	0 0	0 0	0 0	0·0	0·0
8	0 0	0 0	4 0	0·0	0·0
12	0 0	20 0	28 0	8·0	0·0
16	16 0	24 0	32 0	12·0	4·0
20	20 0	40 0	40 0	16·0	8·0
24	24 0	48 0	60 0	20·0	12·0
28	28 0	48 0	60 0	20·0	12·0
32	28 0	52 0	60 0	20·0	12·0
56	28 0	52 0	60 0	20·0	12·0
40	28 0	52 0	60·0	20·0	12·0
44	28 0	52 0	60·0	20·0	12·0

The pathogen showed positive infection of seedlings at all the temperatures under study The total post-emergence seedling mortality was 28% at 20° C, 52% at 25° C and reached the maximum value of 60% at 30° C, but further increase in temperature resulted in considerable fall in mortality (Table 6) It is also clear from the above table that most of the seedlings wilted in early part of the experiment, between 12-24 days, giving indications thereby that young seedlings are more susceptible to the attack of the parasite than the relatively old ones.

TABLE 7

Showing total percentage mortality in Gram.

(Average of 5 pots, each with 5 seeds)

Temperature in Degree Centigrade					C.D at 5% level
20	25	30	34	38	
36 0	56 0	84 0	20 0	12 0	25 1

The above table clearly indicates that the total percentage mortality (pre and post-emergence together) increases from 36% at 20° C to 56% at 25° C and aquires the maximum value of 84% at 30° C, but at still higher temperature ranges a sharp fall is observed. The various temperatures are arranged below in three groups in the sequence of their effectiveness on total percentage mortality

38°C	34°C	20°C	25°C	30°C
12·0	20·0	36·0	56·0	84·0

C. D is 25 1

From the above grouping it is clear that there is no significant difference in total percentage mortality caused at 38° 34° and 20° C. Similar is the case at 20° and 25° C. At 30° C the mortality percentage is maximum and differs significantly from the other two groups

DISCUSSION

The influence of temperature on growth of *Sclerotium rolfsii* has been studied earlier by others both on natural and artificial media, but the same was repeated here primarily to see whether any direct relationship exists between the growth of the parasite and the disease development in guar and gram. Higgins (1927) found 30°-35° C to be the optimum temperature range for the growth of the parasite while Chowdhury (1948) recorded 28° C as the optimum temperature for its growth in case of sclerotial wilt of betel (*Piper betle*) Linear growth on agar medium was most rapid at 30° C (Abeygunawardena and Wood 1957) In the present investigation the growth of the fungus was studied in Czapek's medium at temperatures ranging from 15° to 42° C and it was found that the maximum growth (colony diameter—

105 3 mms) occurred at 30° C. The next lower favourable temperature was 25°C Thus the observations on growth are in conformity with the findings of earlier workers.

It is evident from the results that 25°-30° C is the optimum range for root-rot development in guar and 30° C for maximum wilt development in gram. These observations directly correspond with the growth of the parasite (*Sclerotium rolfsii*) in culture. Similar observations were made by Clayton (1923) in wilt of tomato Jones and Tisdale (1922) in wilt of flax; Chowdhary (1948) in wilt of betel Fisher and Noll (1942) in root-rot of oak and Gupta (1959) in foot-rot of coriander The relationship of soil temperature and disease development in guar corresponds generally with the geographical distribution of the disease so far as is known the disease is prevalent in regions like Western U P in India, and Texas, Arizona and Georgia in U S A. (Singh 1951 Brooks and Harvey 1950 Streets 1948 and *Luttrell 1951*) which are all characterised by a warm summer when the disease is common

Comparing the results obtained in root rot of guar and wilt of gram, it is observed that in both the diseases the percentage emergence of seedlings in the infested pots was lowered as a result of parasitic activity of the fungus in nearly all the soil temperatures studied. In root-rot of guar maximum pre-emergence infection of 24% was noted at 25° C and nearly the same (20%) at 30° C In wilt of gram, 24% infection was recorded at 30° C while only 4% at 25 C The trends observed for post-emergence percentage mortality in the two diseases was almost identical, mortality increasing with rise in temperature from 20° to 30° C, but declines with further rise in soil temperature. It was maximum at 30° C both in guar (52%) and gram (60%) The total percentage mortality (pre and post-emergence together) was maximum (72%) at 30° C in root rot of guar but this did not differ significantly with the mortality figure (56%) at 25° C. In wilt of gram, the total mortality was maximum (84%) at 30° C, differing significantly with the mortality at 25°C. This clearly shows that gram is relatively more susceptible than guar to the attack of *S rolfsii* under similar conditions of soil temperature.

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*Original not seen—Source Abstracts in R. A. M.

INCIDENCE OF *FUSARIUM* WILT OF GRAM (*CICER ARIETINUM* L.) IN RELATION TO SOIL MOISTURE*

S K. CHAUHAN
Botany Department Agra College Agra

Soil moisture has been found to be an important factor in relation to soil borne diseases Garrett (1944) has compiled a data on the effect of soil mosture on soil brone diseases showing that some diseases may be favoured by low soil mosture while others by high mosture values. Subramanian (1950) working on the wilt of cotton in India, showed that this disease is favoured by high soil moisture.

The present paper deals with the effect of soil moisture on the incidence of wilt of gram (*Cicer arietinum* L.) caused by *Fusarium orthoceras* App. & Wr var *ciceri* Padwick.

MATERIAL AND METHOD

A series of pot-culture experiments was conducted to observe the effect of four different soil moisture levels on the incidence of disease. The mosture levels maintained being 10, 15 20 and 25% well within the water-holding capacity of the soil which was determined following Keen-Raczkowski's method as outlined by Piper (1944)

Having determined the water-holding capacity of the soil (39 1%) the various moisture levels were maintained throughout the experimentation by adding required amount of water each day in pre-weighed glazed metal pots together with weighed amount of soil 6 lbs of garden soil of which the water-holding capacity was previously determined was filled in each of the pot of the experiment series

The soil was infested with the pathogen in the usual way In the control series the soil was not infested. Three days after inoculating the soil, five seeds of gram were sown in each pot. The pots were kept in a glasshouse Six replications of pots were maintained throughout for each treatment and its control. When wilting was noticed the number of plants affected on each day was recorded separately for each pot and its treatment The fungus was reisolated from the wilted plants to make sure the cause of wilting In the control none of the plants showed any sign of wilting in any of the treatment under trial. The figures for the total mortality have been analysed statistically and the rate of mortality plotted on a graph for the different treatments Critical difference was calculated as the experiment was significant.

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* A part of the thesis approved for the Ph. D degree of the Agra University

RESULTS

The data of the series dealing with the effect of different levels are presented below

TABLE

Showing Percentage Mortality in Different Moisture Levels

(Figures relate to the average mortality per pot of five plants each)

10% Moisture	15% Moisture	20% Moisture	25% Moisture	Critical Difference
13 33 ±2 08	26 66 ±3 61	36 66 ±4 73	83 33 ±9 08	14 717

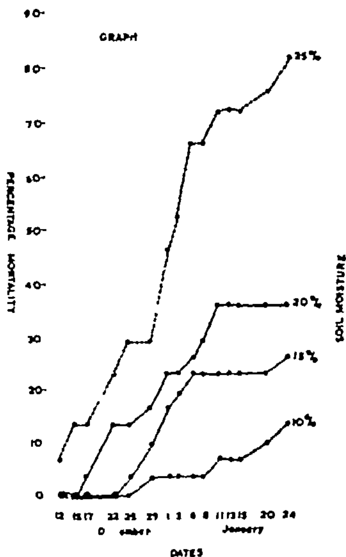
Critical Difference at 5% Level

The above table indicates that by keeping the soil moisture low there is a significant reduction in the percentage mortality The order of reduction in the percentage mortality being 83 33 36 66 26 66 and 13.33 at 25, 20, 15 and 10 % respectively On the basis of the critical differences these figures can be represented as below

10% Soil Moisture	15% Soil Moisture	20% Soil Moisture	25% Soil Moisture
13 33	26 66	36 66	83 33
I		II	III

Further the progressive percentage of mortality has been shown in the graph. It will be seen that in 25% moisture level there is a progressive increase in the disease development until the mortality reaches above 80%. Among the remaining moisture levels 20% seems to be more or less favourable, the disease development being noticed not long after seedlings have been emerged out and increases progressively until mortality is 36%. A further increase is probably limited by some unknown factor and no more mortality occurs. In 15% soil moisture the incidence of the disease is late but the mortality is progressive until it reaches about 26% above which it does not increase. In 10% level of soil moisture, the disease incidence is very late and afterwards too, shows no progressive development, the mortality rising for time and then subsiding and rising again to subside in factors which lead to a very erratic and vacc

Graph shows progressive percentage rate of mortality in different soil moisture level.



CONCLUSION

Vascular wilts caused by various species of *Fusarium* are found to be favoured by high soil moisture level. This point that been supported from time to time by several workers (Tharp & Young 1939, Pontis 1940 and Subramanian 1950) Wilt of gram caused by *Fusarium orthoceras* var *ciceri* is also found to be favoured by high soil moisture contents as seen in the present investigation. If the soil moisture level is very high the prevalence of the wilt diseases may increase in another way i.e., by flooding dispersal, which fact has been pointed out by Wardlaw (1935) in the Pannama disease of Banana. Very

high moisture content may also by reducing soil aeration injure the roots of the host plant and finally produce wilting In the present experiments care has been taken that the moisture level may not become so high as to cause wilting other wise than the pathogen.

Summary

Influence of four levels of soil moisture, well within the waterholding capacity *viz* 10, 15 20 and 25% (on oven dry weight) was observed on the wilt mortality in gram (*Cicer arietinum* L.) caused by *Fusarium orthoceras* App. & Wr var *ciceri* Padwick. The figures obtained on final mortality were analysed statistically and found to be significant, the C. D for the figures being 14 71 The order of reduction in the percentage mortality being 83.33, 36.66, 26 66 and 13.33 at 25 20 15 and 10% soil moisture levels respectively

Acknowledgements

The author is grateful to Prof. S Sinha, Head of Botany Department, Agra College, Agra for his guidance and criticism during the course of investigation. Thanks are also due to U P S. R. C. for grant-in-aid.

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STUDIES ON MINERAL AND PROTEIN METABOLISM*

P P KURIEN

Central Food Technological Research Institute Mysore

A summary of the results of investigations carried out for assessing the nutritive value of poor Indian diets containing varying amounts of rice and/or ragi (*Eleusine coracana*) jowar (*Sorghum vulgare*) or bajra (*Pennisetum typhoideum*) by growth experiments in albino rats and by nitrogen calcium and phosphorus metabolism studies in children is given below

PART A GENERAL INTRODUCTION

In the general introduction (Chapter I) a brief account of the available data on the chemical composition and nutritive value of cereals and millets consumed in India and other parts of the world is given The proteins of cereals and millets are in general, deficient in lysine and one or more of the other essential amino acids. All cereals and millets are low in calcium except ragi which is an unusually rich source of this nutrient. Studies on the nutritive value of diets based on different cereals and millets have shown that the overall growth promoting value of poor diets based on millets like ragi, jowar and bajra is higher than that of poor rice diet. Studies so far carried out on the metabolism of nitrogen calcium and phosphorus in human subjects have been confined to diets based on rice and wheat. No investigations have been reported in children with diets based on ragi, jowar and bajra.

PART B. INVESTIGATIONS ON RAGI AND RAGI DIETS

Part B contains results of investigations undertaken to study the nutritive value of poor vegetarian diets containing rice and/or ragi by the rat growth method and the metabolism of nitrogen calcium and phosphorus in children on poor Indian diets containing rice and/or ragi.

The introductory Chapter (Chapter II) is a review of the work done so far by various workers on the chemical composition and nutritive value of ragi and ragi diets

In Chapter III the results of investigations on the chemical composition of a few varieties of ragi are presented.

The distribution of protein, calcium and phosphorus between the husk and endosperm was studied. The husk was separated from the grain by a

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*It is summary of the Thesis submitted and approved for the Degree of Doctor of Philosophy of Agra University Agra in the year 1961

wet processing technique. The husk accounted for about 13 per cent of the grain, and contained about 28 per cent of the total protein, 49 per cent of the total calcium and 14 per cent of the total phosphorus present in the whole grain. The poor digestibility of the proteins and the low absorption of calcium from ragi diets observed by different workers have been discussed.

An estimation of the essential amino acid composition of the proteins of ragi by chemical and chromatographic methods showed that ragi proteins are deficient in lysine, confirming the observations made by other workers.

Animal Experiments

The nutritive value of poor rice diets wherein rice had been replaced to the extent of 25 50 or 100% by ragi was studied by the rat growth method. The results showed that replacement of rice in the poor rice diet by ragi even to the extent of 25 per cent resulted in an increase in the overall nutritive value of the diet as judged by the growth of rats. A further increase in the ragi content of the diet did not produce any appreciable increase in the rate of growth of rats.

The haemoglobin and R. B C contents of the blood of rats fed on diets containing 25 50 or 100% of ragi were significantly higher than those of rats fed on the rice diet. No significant differences were, however, observed in the serum protein levels in the different groups.

No significant difference was observed in the mean nitrogen content of the livers of rats fed on the different diets. The fat content of the livers of animals fed on diets containing 25 50 or 100 per cent ragi was slightly higher than that of the livers of rats fed on the rice diet.

Metabolism in Children

The effect of partial (25 or 50 per cent) or complete replacement of rice in the poor rice diet by ragi on the metabolism of nitrogen, calcium and phosphorus in eight children (girls) aged 9-10 years was studied.

The daily intake of nitrogen by the children was nearly of the same order varying from 4 41 to 4.51 g (equivalent to about 28 g. of protein) on the different diets. The apparent digestibility coefficients of the proteins were 71 67 64 and 53 per cent and the mean daily retentions of nitrogen were 1 48 1 19 0 89 and 0.52 g respectively for the rice diet and the diets in which rice had been replaced at 25 50 or 100% levels by ragi. All the subjects were in positive nitrogen balance.

The mean daily intakes of calcium on the different diets were 239 471, 693 and 1151 mg and the retentions were 52 106, 175 and 276 mg. on the rice diet and the diets containing 25 50 or 100 per cent levels of ragi. The retentions of calcium on the diets containing ragi were significantly higher than those on the rice diet.

The mean daily intakes of phosphorus were 464 577 680 and 887 mg and the retentions were 117 165 125 and 195 mg respectively on the rice diet and the diets containing 25 50 or 100 per cent levels of ragi.

Replacement of rice in poor rice diet by ragi helped to overcome the calcium deficiency of the rice diet, as shown by the growth of rats and retention of calcium by children fed with rice-ragi diets.

PART C. INVESTIGATIONS ON JOWAR AND JOWAR DIETS

Part C of the thesis contains the results of investigations on the nutritive value of poor vegetarian diets containing rice and/or jowar by the rat growth method, and the effect of replacing rice in poor Indian diets by jowar on the metabolism of nitrogen calcium and phosphorus in children.

The available information on the chemical composition and the nutritive value of jowar and jowar diets is reviewed in Chapter VI

Chemical Composition of Jowar

The chemical composition and the distribution of protein calcium and phosphorus between the husk and endosperm of three varieties of jowar were determined. A wet processing technique was adopted to separate the husk and the germ from the endosperm followed by a gravitational method to separate the husk from the germ. The husk accounted for 10 3 per cent of the whole grain and contained 17 6 per cent of the total protein, 20.2 per cent of the total calcium and 12 7 per cent of the total phosphorus present in the whole grain

Assay of the essential amino acid composition of jowar proteins by chemical and chromatographic methods have confirmed the earlier observations that the proteins of jowar are deficient in lysine.

Animal Experiments

The nutritive value of poor vegetarian diets based on rice and/or jowar was studied by the rat growth method. Substitution of rice in poor rice diet by jowar at 25 50 or 100 per cent levels did not affect to any significant extent the overall nutritive value of the diet as judged by the growth of rats.

There was no significant difference in the haemoglobin and total serum protein content of the blood of rats fed with the different diets. The R.B.C. counts of the blood of rats fed on 25 and 50% jowar diets were, however slightly higher than those of rats fed on the rice diet.

The average fat content of the liver of rats fed on the different diets fell within the normal range (3 65–3 95 per cent) The average protein content of the livers of the animals fed on 100 per cent jowar diet was, however less than those fed on the other diets.

Metabolism in Children

The effect of replacing rice in a poor Indian diet by jowar to the extent of 25 50 or 100% on the metabolism of nitrogen, calcium and phosphorus was studied in seven children (boys) aged 10-11 years.

The daily intakes of nitrogen by the experimental subjects varied from 6.34 to 6.91 g on the different diets. The apparent digestibility of the proteins in the diet decreased with the increase in the amount of jowar in the diet. The apparent digestibility of the proteins were 74 7 69.3 63 7 and 55.4 per cent and the mean daily retentions were 1.80 1 55 1.28 and 0.88 g respectively on the rice diet and on the diets in which rice had been replaced by 25, 50 or 100 per cent of jowar All the subjects were in positive nitrogen balance on all the diets.

The mean daily calcium intakes were 355 381 410 and 441 mg. and the retentions were 123 109 97 and 74 mg respectively on the rice diet and the diets in which rice had been replaced by 25 50 or 100 per cent of jowar. All the subjects were in positive calcium balance on the different diets

The mean daily intakes of phosphorus were 744 835 928 and 1091 mg and the retentions were 169 204 233 and 309 mg respectively on the rice diet and the diets in which rice had been replaced by 25, 50 or 100 per cent of jowar All the subjects maintained positive phosphorus balance on all the different diets.

The results of these investigations have shown that rice in a poor Indian diet can be substituted partially (25% level) by jowar without affecting to a significant extent the overall nutritive value of the diet.

PART D INVESTIGATIONS ON BAJRA AND BAJRA DIETS

Investigations similar to those undertaken in the case of ragi and jowar were carried out with bajra to study the effect of replacing rice in poor rice diet by bajra at different levels (25 50 or 100% levels) on the growth and composition of the blood and liver of rats and the metabolism of nitrogen, calcium and phosphorus in children. These experiments are presented in Part D of the thesis.

A review of the available informations on the chemical composition and nutritive value of bajra and bajra diets are presented in the introductory chapter (Chapter X)

Chemical Composition of Bajra

The chemical composition and the distribution of protein, calcium and phosphorus between the husk and endosperm of three different varieties of bajra were determined The separation of the husk from the endosperm was effected by a wet processing technique The husk accounted for 11.8 per cent

of the whole grain and contained 11.5 per cent of the total protein, 36 0% of the total calcium and 14 per cent of the total phosphorus present in the whole grain.

Assay of the essential amino acid composition of the bajra proteins by chemical and chromatographic methods have confirmed the earlier observation that the proteins of bajra are deficient in lysine

Animal Experiments

The effect of partial or complete replacement of rice in poor vegetarian diet by bajra partially (25 or 50) or completely on the growth and composition of the blood and liver of rats was studied. The results showed that substitution of rice in poor rice diet by bajra resulted in a slight increase in the nutritive value of the diet as judged by the growth of rats.

There were no significant differences in the haemoglobin R. B C. and the total serum protein contents of the blood of rats fed on the different diets.

The average fat contents of the livers of rats fed on the different diets were within the normal range. The average protein content of the livers of the rats fed on complete bajra diets was significantly less than that of rats fed on the other three diets.

Metabolism in Children

The effect of replacing 25% 50% or 100% of the rice in poor rice diets by bajra on the metabolism of nitrogen calcium and phosphorus was studied in eight children (boys) aged 11 12 years.

The daily intake of nitrogen on the different diets ranged from 6.92 to 8.67 g. The apparent digestibility coefficients of the proteins were 75 73, 64 and 53 per cent. The daily retentions of nitrogen were 2.02, 1.87 1 49 and 1 11 g. respectively on the rice diet and the diets in which rice had been replaced to the extent of 25, 50 or 100% by bajra. All the subjects were in positive nitrogen balance.

The mean daily intakes of calcium on the different diets were 352, 378, 418 and 479 mg. and the retentions were 119 117 113 and 92 mg respectively on the rice diet and the diet in which rice had been replaced at 25% or 50% or completely by bajra. All the subjects were in positive calcium balance.

The mean daily intakes of phosphorus were 726 867 1029 and 1346 mg. and the retentions were 162, 224 297 and 356 mg respectively on the rice diet and the diets in which 25 50 or 100% of rice had been replaced by bajra. All the subjects were in positive phosphorus balance

The results of these investigations have shown that bajra can be used as a partial substitute for rice in poor rice diets.

Appendix

INVESTIGATIONS ON "*Paushtic Atta*"

The results of investigations on the effect of replacing wheat in the diet by *Paushtic atta* (a blend of whole wheat flour 75 parts tapioca flour 17 parts and low fat groundnut flour 8 parts) on the growth and metabolism of nitrogen calcium and phosphorus in children are presented in the Sections I and II of the Appendix.

SECTION I. GROWTH STUDIES ON CHILDREN

The effect of replacing wheat (forming 50% of the cereals in the diet) in a poor Indian diet by a blend of whole wheat flour (75 parts), tapioca flour (17 parts) and low fat groundnut flour (8 parts) (*Paushtic atta*) on the growth and nutritional status of children was studied. A feeding experiment extending over a period of three months was carried out on thirty boys belonging to the ag group 6-12 years. Th subjects were housed in a boarding home. The children were paired according to initial height, weight and nutritional status and the members of each pair were allotted at random to the whole wheat flour diet or *Paushtic atta* diet and fed.

Values for the height, weight nutritional status haemoglobin level and red blood cell count were obtained at the beginning and end of the experiment for the subjects in the two groups. No significant difference was observed in the increase in the height, weight, red blood cell count, haemoglobin and nutritional status of the subjects in the two groups.

SECTION II. METABOLISM IN CHILDREN

The effect of replacing whole wheat flour which forms 50 per cent of the cereals in a poor Indian diet by *Paushtic atta*" on the metabolism of nitrogen, calcium and phosphorus was studied in eight pairs of children (boys) aged 11 12 years.

The mean daily intakes of nitrogen were 9·78 and 9·97 g and the apparent digestibility coefficients of the proteins were 74 5 and 75·8 per cent respectively on the whole wheat flour and *Paushtic atta* diets. All the children were in positive nitrogen balance on both the diets. The daily retentions of nitrogen on whole wheat and *Paushtic atta*' diets were 1·61 and 1·90 g respectively.

The mean daily intakes of calcium on the whole wheat flour and *Paushtic atta* diets were 694 and 702 mg All the children were in positive calcium balance. The mean daily retentions of calcium were 102 and 113 mg respectively on the two diets.

The mean daily intakes of phosphorus were 1410 and 1350 mg and the retentions were 395 mg and 404 mg respectively on the whole wheat flour diet and *Paushtic atta* diets

The results of these investigations show that a blend of 75 parts of whole wheat flour 17 parts of tapioca flour and 8 parts of low fat groundnut flour called *Paushtic atta* is as good as whole wheat flour if not better in maintaining the height, weight and nutritional status, and in promoting adequate retentions of nitrogen, calcium and phosphorus in children

The investigations have shown the possibilities of blending wheat flour with a blend of groundnut flour and tapioca flour which are available in plenty in the country and thus augmenting the supplies of wheat in the country by 25%

ACKNOWLEDGEMENT

My thanks are due to Dr M. Swaminathan D Sc. F N I for his valuable guidance and Dr V Subrahmanyan D Sc. F N I for his keen interest in the work and Dr Narayana Rao, M. Sc., Ph D for the help in the preparation of the thesis.

STUDIES ON THE INNERVATION OF THE HEART*

RAMESH CHANDRA HARITWAL
Zoology Department Science College Raipur M P

In spite of the voluminous literature on the anatomy and the physiology of the vertebrate heart, it is surprising to note that studies on the innervation of the avian heart have been few and among mammals reference is mostly available on the innervation of the human heart only

This notable gap in our knowledge about the morphology of the con necting tissue of the vertebrate heart has been mainly responsible for the continuous existence of the controversy that unfortunately arose since early times between the protogonists of myogenic and neurogenic theories of cardiac conduction. Of the host of workers who studied the cardia connecting tissue the majority devoted attention only to the histology and the physiological properties of the various types of muscle in the vertebrate heart. Even in this limited field, the literature is full of cotroversial statements and varied results

In support of the neurogenic theory of cardiac conduction a few of the earlier investigators (Stannius, 1852 Gaskell, 1886 Dogiel and Archangelsky 1906 Dogiel, 1907 and Imchantizky 1908 and 1909) who confined their observation on lower vertebrates only laid stress on the role of nerves and ganglia of the heart for initiation and conduction of the stimulus of contraction.

The controversial statements and incomplete studies of the previous investigators necessitated further studies on the morphology of the conduction system specially the innervation of the heart in birds and mammals.

From the extensive literature it is clear that in contrast with the very numerous investigations that have been made on the muscular conduction system of vertebrate heart, the studies on the structure and the distribution of the nerves that supply the heart and its conducting tissue have been very few Some of the investigators who tried to fill up this gap in our knowledge paid attention only to a few selected types and in these also only selected regions of the heart were examined. To the best of author s knowledge no authentic detailed description is available in the literature about the innervation of the avian heart. Among mammals the study has been mostly confined only to the human heart. In the present investigation therefore, the innervation of the heart and its conducting tissue has been studied in three avian and three mammalian species.

The innervation of the heart has been studied in three avian species namely the common Indian fowl, *Gallus gallus* the blue rock pigeon *Columba*

*This is an abstract of the thesis submitted and approved for the Ph D. degree of the Agra University Agra in the year 1960.

luna intermedia the house sparrow *Passer domesticus* and also in three mammalian species viz. the albino rat, the shrew *Suncus murinus* and the bat, *Rhinopoma kinneri*.

The nerve supply of the heart, both in birds and mammals, is complex and like other visceral structures is mainly from sympathetic and parasympathetic components of the cervical autonomic nervous system.

It has been observed that nerves contain both efferent and afferent fibres which are involved in important reflexes and which are influenced in their activities by the stimuli received from many quarters.

The vagus nerve is the main source of parasympathetic contribution to the heart. The nerves arise both in the neck and the thorax and are variable in number and movement.

The middle cervical sympathetic ganglion though present, is not very much defined and distinct in mammalian hearts.

The previous investigators reported the absence of the middle cervical sympathetic ganglion in birds heart The present study has shown that it is present though very much reduced in size

In mammals a loop of nerve ansa-subclavia is formed by a branch arising from the middle cervical ganglion and encircling the subclavian artery of its side

Ansa-subclavia is absent in the heart of birds.

The thoracic nerves arise, in mammals from the first four thoracic ganglia, and in birds from the first five thoracic ganglia. Neither in birds nor in mammals the sixth or seventh thoracic ganglia were found to give rise to cardiac nerves

The cardiac plexus has been observed to be formed by the coalescence of all the nerves coming to the heart Almost all previous investigators divided the cardiac plexus into-dorsal and ventral, caudal and cranial and anterior and posterior portions. The present study shows that the cardiac plexus both in birds and mammals, is indivisible.

In confirmity with the observation of the previous investigators, small intrinsic ganglia have been observed in the subepicardial and myocardial tissues of the heart. Small ganglia are also located in the auricular appendages, around the roots of great vessels, in the interatrial septum, in the atrioventricular sulcus and in the nerve bundles of sinuatrial and atrioventricular nodes.

Contrary to the findings of the previous investigators difference in the size of the various ganglia lying in the different regions of the heart, either in birds or mammals, was not noticed.

Nerve branches in the left atrium are very few in number and mainly come from coronary aortic and pulmonary nerves.

The right atrium is rich in its nerve supply than the left atrium. Its posterior anterodorsal and anteroventral portions have been found to be supplied by coronary sinus and aortic nerves respectively

The distribution and arrangement of nerve firbres and ganglion cells within the right atrium has been found to resemble that described by Glomset and Glomset (1940) in dog and man.

Nerve plexus are much more extensive in the right atrium of the albino rat than in that of any other mammal or bird.

The main source of nerve supply to the ventricles is from the coronary nerve. All the portions of the ventricles do not receive a uniform supply of the nerve fibres. The anterior portion of both the ventricles, the atrioventricular sulcus and the interventricular septum are rich in nerve fibres while the posterior region of both the ventricles is a very poor recipient of nerve fibres Ganglia are not commonly found in the ventricle.

The sinuatrial node is almost invariably present in all the species of birds and mammals. In mammalian heart this node is extensively supplied with the nerve fibres to form a plexus outside as well as inside its body

The nerve fibres supplied to the heart are mostly postganglionic in all the avian and mammalian species.

The main nerve supply to the atrioventricular node has been found to be from the aortic nerve

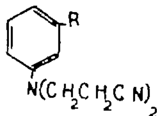
The concept of muscular conduction system in the vertebrate heart has been reaffirmed and at the same time evidence has been presented to show that there is a sound anatomic basis for the neurogenic theory of cardiac conduction.

THE CHEMISTRY OF NN BIS-2-CYANOETHYL DERIVATIVES OF PRIMARY AROMATIC AMINES AND THE HETEROCYCLIC KETOAMINES DERIVED FROM THEM*

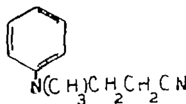
ABRAHAM THOMAS
St John's College Agra †

The addition of acrylonitrile to compounds containing reactive methylene groups is known as 'Cyanoethylation. Monocyanoethylation of aromatic amines has been known for some time and the literature on the subject is extensive. Dicyanoethylation of aromatic amines has been achieved only recently Braunholtz and Mann[3] have studied the cyanoethylation of aniline under the influence of various inorganic catalysts and have developed an excellent method for the dicyanoethylation of aromatic amines using acrylonitrile freshly prepared cuprous chloride and glacial acetic acid

The properties of the bis-cyanoethyl derivatives have not been investigated by the above workers in detail. *In part I of this dissertation is embodied* the preparation of NN-bis-2-cyanoethyl derivatives of certain primary aromatic amines and a study of their chemistry For the work described in this dissertation dicyanoethyl derivatives of several aromatic amines were needed particularly those of aniline and m-toluidine (I R=H or CH_3) respectively



(I)



(II)

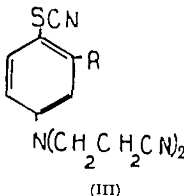
For this the method adopted was that described by Braunholtz and Mann[4] By this method aniline *m*-toluidine and *p*-toluidine were dicyanoethylated. N Methylaniline was also cyanoethylated and N 2 cyanoethyl-N-methylaniline (II) was prepared, according to the method recommended by Allison, Braunholtz and Mann[5] Attempts to dicyanoethylate *m*-chloraniline and *p*-thiocyanoaniline were unsuccessful. However by a different route referred to later it was possible to prepare NN-*bis* 2-cyano-

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* Summary of the thesis submitted for the Degree of Doctor of Philosophy t the University of Agra.

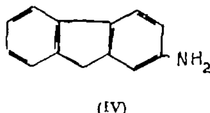
† Present address St. Augustine's College Cape Coast, Ghana.

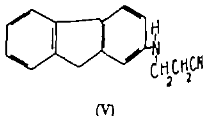
ethyl-p-thiocyanoaniline and NN-*bis* 2'-cyanoethyl-4-thiocyano-*m*-toluidine (III R=H or CH_3)



(III)

In an attempt to dicyanoethylate 2 aminofluorene (IV), the product isolated was N 2 -cyanoethyl 2-aminofluorene (V)

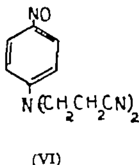


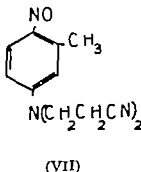


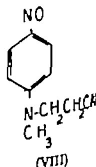
(IV) (V)

The role of cuprous chloride in the cyanoethylation of 2-aminofluorene has been investigated. Whether the product obtained is carcinogenic or not is under investigation, since several derivatives of 2-aminofluorene are notorious carcinogenic agents

Bis 2 -cyanoethylaniline and its *m* toluidine homologue behave as typical dialkylanilines in that they form green crystalline p-nitroso derivatives (VI) and (VII) respectively p-Nitroso derivative of N-2'-cyanoethyl N methylaniline (VIII) has been prepared now



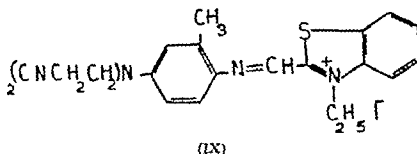




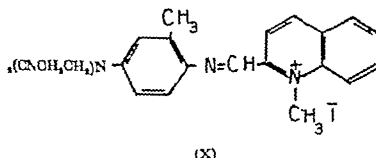
(VI) (VII) (VIII)

The compounds (VI) (VII) and (VIII) were condensed with a number of compounds containing reactive methylene groups. The reactive methylene

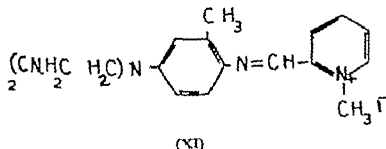
compounds studied were 2 4-dinitrotoluene 5-methylacridine 2 phenylindole, dibenzoyl methane α-picoline methiodide quinaldine methiodide, 2-methylbenzthiazole ethiodide and Fischer's base hydriodide. The anils obtained are highly coloured lustrous crystalline compounds The structures of some of them are listed below



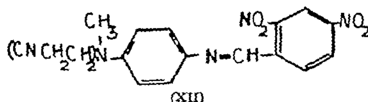
(IX)



(X)



(XI)

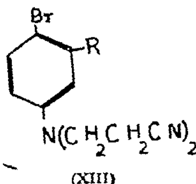


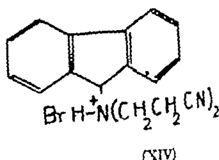
(XII)

Photosensitising properties of one of them has been investigated by I.C.I Ltd. Dye-stuffs Division England. Dye (X) gives no optical sensitisation but severe desensitisation with fog

It was attempted to reduce the C-Nitroso group into amino group by reduction in acid media with tin The product isolated is expected to be the stannichloride complex of the expected amine which needs confirmation.

Another reaction of the bis-cyanoethyl derivative studied is the nuclear bromination using N bromosuccinimide or bromine-dioxane complex. Ittyerah and Mann[8] have brominated NN *bis* 2-cyanoethylaniline by N bromosuccinimide and prepared the p-bromoderivative (XIII R=H).



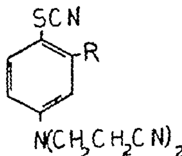


(XIII) (XIV)

This reaction is of great significance as the dicyanoethylation of p bromoaniline is an extremely difficult process. Nuclear bromination has been now extended to (I R=CH$_3$) by N-bromosuccinimide and bromine dioxane complex. The latter has been found to be a better reagent for bromination in so far as the yields are better and the purification of the product easier *p* Bromo derivative (XIII R=CH$_3$) has also been prepared

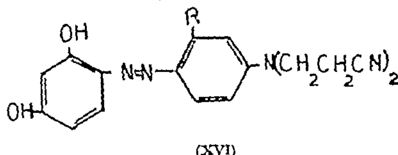
In an attempt to extend the nuclear bromination to NN-*bis* 2-cyanoethyl 9-aminofluorene only the hydrobromide could be isolated (XIV). The fact that NN-*bis* 2-cyanoethyl 9-aminofluorene forms such salts was further demonstrated by the preparation of its hydrochloride.

Aromatic thiocyano compounds provide a route to the preparation of phenols thiophenols, sulphonic acids and isothiocyanates In the dissertation is described the thiocyanation of the dicyanoethyl derivatives of aniline and m toluidine using ammonium thiocyanate and their formation of their *p* thiocyano derivatives (XV R=H or CH$_3$)



(XV)

J. M. Tedder[1] has developed a method by which diazonium groups can be directly introduced into the aromatic nucleus by one step without using the classical method of nitration reduction to the amine followed by diazotisation. The bis-cyanoethyl derivatives (I R=H or CH_3) have now been diazotised by the new method and coupled with resorcinol to give azo-dyes (XVI R=H or CH_3)



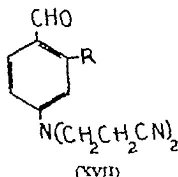
(XVI)

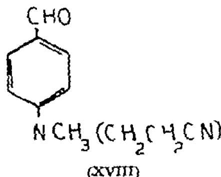
Tertiary aromatic amines couple readily with diazotised primary amines This reaction has now been extended to bis-cyanoethyl derivatives with the formation of azo dyes.

p-Dialkylaminobenzaldehydes are important intermediates in the synthesis of cyanine dyes and a host of other compounds of great importance.

Due to the similarity of the bis-cyanoethyl anilines to dimethylaniline in chemical properties it was attempted to introduce a formyl group in the aromatic nucleus of these compounds Formylation by the method recommended by R. Adams and H Coleman[1] was ineffective However formylations of (I R=H or CH_3) and (II) have now been achieved by using both N methyl formanilide and dimethyl formamide, and the *p*-formyl derivatives (XVII R=H or CH_3) and XVIII prepared. These aldehydes have been characterized by the preparation of suitable derivatives.

It has been found that formylation by dimethyl formamide is better in so far as it is simpler gives better yields, and the product obtained is purer





(XVII) (XVIII)

The aldehydes (XVII R=H or CH_3) were studied in detail.

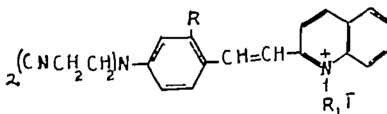
The condensation of aldehydes with amino compounds like hydrazine, *p*-aminobenzoic acid anthranilic acid, β-naphthylamine has been studied. The Schiff's bases isolated are highly crystalline and coloured compounds.

The reactions of the aldehydes with reactive methylene compounds like 2:4-dinitro toluene di-ethyl malonate malonic acid, malono nitrile, ethyl cyanacetate, benzyl cyanide, di-benzoyl methane 5-methyl acridine, α-picoline methiodide γ-picoline methiodide, quinaldine methiodide, 2-methyl benzthiazole ethiodide, Fischer's base hydriodide etc. are also described in the dissertation.

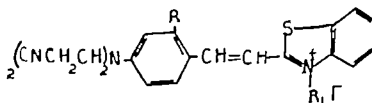
It is found that in the presence of organic bases like pyridine or piperidine as catalysts the aldehydes readily condense with malonic acid, its derivatives as also with several compounds with reactive methylene groups.

A new series of cyanine dyes have been obtained from the aldehydes (XVII R=H or CH_3) by condensation with heterocyclic bases mentioned above and their photosensitising properties, investigated by arrangement with the Imperial Chemical Industries Ltd. England, Dyestuffs Division.

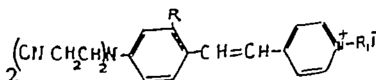
These dyes are highly coloured lustrous crystalline compounds. Their colour in solution is discharged on addition of acids and regained on addition of alkali. Listed below are the formulae of some of the dyes synthesised.



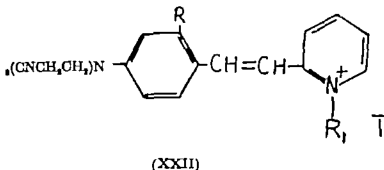
(XIX)



(XX)



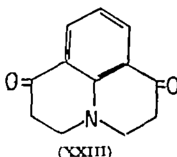
(XXI)



(XXII)

In the above formulae R = CH_3 or H and R_1 = CH_3 or C_2H_5

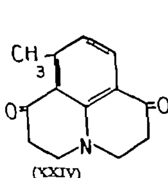
Cyclisation of the di-cyanoethyl derivatives in presence of anhydrous aluminium chloride and chloro benzene and subsequent hydrolysis yield hetero cylcic systems of the type (XXIII)



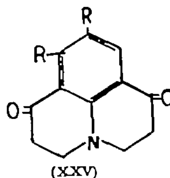
(XXIII)

Such hetero cyclic systems in which nitrogen forms a bridge head atom is of considerable interest as such systems are found in a wide variety of natural products. Braunholtz and Mann[24] and later Ittyerah and Mann[24] have prepared such systems and studied the reactions of these compounds. The work that is embodied in the second part of the dissertation is an extension of the work initiated by the above workers.

For the present work described in detail in the dissertation, two hetero-cyclic ketoamines (XXIII) and (XXIV) were specially needed and this was prepared by the method recommended by Braunholtz and Mann



(XXIV)



(XXV)

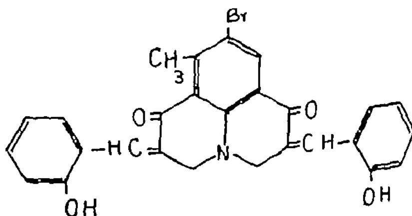
It is now found that the yields obtained in th cyclisation [reaction depend to a large extent on the quality of aluminium chloride used. Very pure specimens are not so effective as technical quality ones. In practice it is found that when very pure aluminium chloride is used, the addition of a trace of ferric chloride helps

The application of several reactions to these hetero cyclic systems has been investigated.

(I) Bromination of the dioxojulolidines.

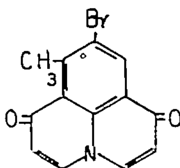
Attempts by Ittyerah and Mann[8] to prepare (XXV R=H R_1=Br) by the cyclisation of p-bromo-NN-bis-2-cyanoethylaniline was unsuccessful. So the above authors tried the nuclear bromination of 1 6-dioxojulolidine by N-bromo succinimide and were successful in isolating (XXV R=H and R_1=Br). Now the bromine dioxane method has been extended, and it has been found that though the yield of the product is not better purification of the product is easier

7 Methyl 1 6-dioxojulolidine has not been brominated so far It has now been achieved and the diketo amine (XXV R=CH_3 R_1=Br) prepared. Several derivatives like the 2 4-dinitrophenyl hydrazone, the bis-phenyl hydrazone, the dioxime, the bis-salicylidene derivative (XXVI) have now been prepared.

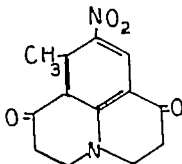


(XXVI)

An attempt to prepare tri-cyclic systems of the type (XXVII) by the direct bromination of 8-bromo-7 methyl 1 6 dioxojulolidine followed by dehydrobromination failed The product was a polymeric material melting over a wide range probably a mixture of bromo derivatives.



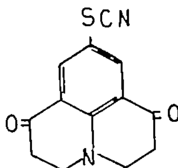
(XXVII)



(XXVIII)

(ii) Nitration of 1 6-dioxojulolidine has been done by earlier workers. This has now been extended to 7-methyl 1 6-dioxojulolidine and the 7-methyl-8-nitro-1 6-dioxojulolidine (XXVIII) prepared. Several derivatives like the dioxime (bright yellow crystals) bis phenyl hydrazone (deep red crystals) and 2 5-bis-*p*-dimethylaminobenzylidene-7 methyl-8-nitro-1 6-dioxojulolidine have now been prepared and characterised.

(iii) Attempts to prepare 1 6-dioxo-8 thiocyanojulolidine (XXIX) by the biscyanoethylation of *p*-thiocyanoaniline and subsequent cyclisation were futile. The only way left for the preparation of (XXIX) was by the direct thiocyanation of 1 6-dioxojulolidine and this has now been achieved using ammonium thiocyanate and bromine. These thiocyano compounds are interesting starting materials for thiophenols, sulphonic acids, and isothiocyanates.

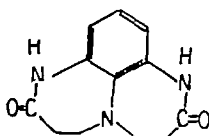


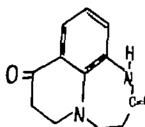
(XXIX)

(iv) Formylation and benzoylation of the 8th position of 1 6-dioxojulolidine were unsuccessful.

(v) Application of Clemmensen s method of reduction to the heterocyclic ketoamines met with no success.

(vi) Ittyerah and Mann[8] have reported that the application of the Schmidt reaction to 1 6-dioxojulolidine yielded the bis-lactam (XXX) while 7 9-dimethyl-1 6-dioxojulolidine did not undergo this reaction. This is obviously due to the fact that carbonyl groups in the latter are

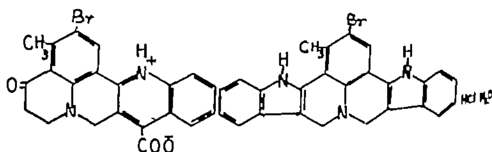




(XXX) (XXXI)

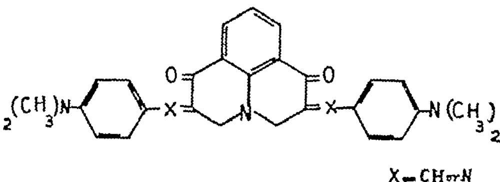
sterically hindered by the methyl groups at positions 7 and 9 The author therefore expected that 7-methyl 1 6-dioxojulolidine would yield the monolactam (XXXI) Several conditions were tried. Under ordinary conditions of the experiment, unreacted 7-methyl 1 6-dioxojulolidine was isolated and under forced conditions, an intractable gum was the only product obtained.

(vii) Pfitzinger Reaction and Fischer's indolisation have been applied to the diketoamine (XXV $R=CH_3$ $R_1=Br$) 8-Bromo-7-methyl-6-oxo-quinolino (2 : 3 1 : 2) juloline carboxylic acid (XXXII) and the monohydrated monohydrochloride of the diindolo (2 3 1 : 2) (3 2 5 6) 8-bromo-7-methyl juline (XXXIII) were prepared.



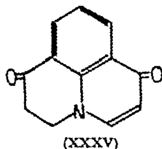
(XXXII) (XXXIII)

(viii) Some of the heterocyclic ketoamines, the chemistry of which forms the subject matter of the second part of the dissertation have been used in the preparation of mero-cyanines of the type (XXXIV)

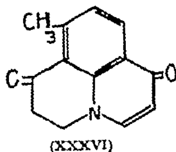


(XXXIV)

Ittyerah and Mann[5] have reported the preparation of 1 : 6-dioxo-iso-juloline (XXXV) by the action of palladised charcoal in boiling ethylene glycol



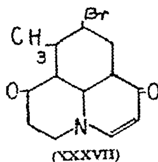
(XXXV)



(XXXVI)

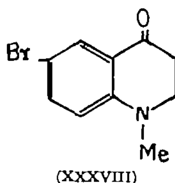
or cymene. This has now been extended to two more dioxojulolidines and the iso-julolines (XXXVI) and (XXXVII) prepared.

Several derivatives like the hydrobromide, picrate, monophenyl hydrazone and the bis-perchlorate have been prepared to establish the structure of these products

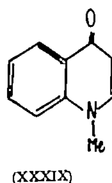


(XXXVII)

Formation of the iso-juloline (XXXVII) is interesting in view of the fact that under similar conditions of dehydrogenation the bromine atom of the oxoquinoline (XXXVIII) is split off from the molecule leading to the formation of (XXXIX)



(XXXVIII)



(XXXIX)

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1 Adams & Coleman *Org Synth Coll.* Vol. I P 214 John Wiley publication, 1951
2 Allison, Braunholtz & Mann *J Chem Soc* 1954 403
3 Braunholtz & Mann *J Chem Soc* 1953 1817 24
4 Braunholtz & Man *J Chem. Soc* 1952 3046-51
5 Ittyerah & Mann, *J Chem Soc* 1958 467
6 Ittyerah & Man *J Chem Soc* 1956 3179
7 Tedder J M *J Chem Soc* 1957 4003.

STUDIES IN SOME ESSENTIAL OILS

MUNISHWAR CHANDRA NIGAM
Essential Oil Research Centre Harcourt Butler Technological Institute Kanpur

The thesis records the investigations on some Indian Essential Oils In the course of the work some new compounds were isolated in pure form and studied to elucidate their structures. Studies on economic production of rose oil and the keeping quality and preservation of rose flowers prior to distillation were also undertaken. Two semi-micro methods (i) Spectrophotometric estimation of geraniol and (ii) Chromatographic estimation of stearoptenes in rose oils were also standardised. Following is the chapterwise summary of the thesis.

Chapter I Indian Rose Oils Part I Production of Rose Oil and preservation f Rose Flowers prior to Distillation.

Fresh flowers of Rose damascena were distilled in an improved type of still with twice their weight of water in such a way that the distillation started after 1¼ hours and a distillate equivalent to 70% 65% 60% 55% and 50% were collected in the first 1½ hours of distillation in different sets of experiments. The distillates were further concentrated to ⅛th of their original volume and the oil found floating on the surface of the concentrates was separated by decantation As a result of these studies it was found that maximum yield of oil was attained if the rate of distillation was so adjusted that a distillate equivalent to 60-65% of the weight of fresh flowers were collected in the first 1½ hours of distillation. The yield of oil under these conditions was found to be 0·022-0·024 % on the weight of fresh flowers.

Experiments were also conducted to study the keeping quality of rose flowers and preservation of oil in them. It was found that an appreciable amount of oil is lost if the flowers were kept in shade even for 4 hours after plucking The experiments were conducted with the three common Indian varieties of roses viz R damascena, R. Edward and R. Teplitz. The loss of oil in four hours was 48·18% 66·92% and 26·67% respectively

Further experiments were conducted on the preservation of oil in rose flowers by dipping them in common salt solutions. As a result of these studies it was found that if the flowers were immersed in a 5% salt solution, no appreciable loss could take place even after 24 hours.

Concretes, absolutes and steam volatiles were prepared from f esh rose flowers and studied for their physico-chemical properties

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Abstract of the thesis submitted for the degree of Doctor of Philosophy of the Agra University

Chapter II Indian Rose Oils Part II Examination of Essential Oil from the Flowers of Rosa damascena.

The essential oil of Rosa damascena was obtained in a yield of 0·02 by hydro-distillation of fresh flowers The oil had a very sweet and refreshing odour On detailed chemical examination, it was found to contain

Phenyl ethyl alcohol 20·77% citronellol 18 30% geraniol 10·90%, nerol 2 5% phenyl ethyl acetate 1 58% geranyl acetate 1·2% geranyl tiglate 1·22 % n-nonyl aldehyde 1 50%, carvone 1·05% and citral 1·48%.

The oil was found to contain 20·25% of stearoptenes also. The stearoptenes were composed of

Eicosane 3 15% heneicosane 5 82% docosane 6·05% and tricosane 1·75%.

Chapter III Indian Rose Oils Part III Estimation of Rose oil Components.

(i) *Spectrophotometric Estimation of Geraniol*
(ii) *Chromatographic Estimation of Stearoptenes*

Two semi micro methods for estimating geraniol and stearoptene were developed. The former method is based on the formation of a coloured complex of geraniol with an alcoholic hydrochloric acid solution of phloroglucinol The formation of coloured complex has been attributed to the presence of an allyl or substituted allyl system in geraniol and certain other compounds where such a reaction takes place. The only other rose oil component which is capable of giving a similar reaction and is present in an appreciable quantity is geranyl acetate. The visible spectra of geraniol and geranyl acetate complexes showed maxima respectively at 550 mμ and 540 mμ. It was found that though the value of λ max is 540 mμ in case of geranyl acetate, both geraniol and geranyl acetate obeyed Beer Lambert Law at 550 mμ upto a molar concentration of 0 08 M Hence it was possible to estimate quantitatively the amount of geraniol present in rose oils by applying a suitable correction for geranyl acetate The method developed was verified in the case of synthetic mixtures and Indian rose oils.

A method for quantitative estimation of stearoptenes in rose oils was also developed. The method is based on the fact that the stearoptene being straight chain saturated hydrocarbons would come out most easily and rapidly through a column filled with Grade I alumina when diluted with petroleum ether 1 0 g of the oils were chromatographed over a column packed with 50 g of grade I alumina and eluted with petroleum ether Fractions of 5 ml each were collected, evaporated and weighed. After a study of chromatogram it was proposed to cut 70 ml of eluate for estimating the stearoptene present.

The proposed method was found to conform with usual method has verified in rose oil samples of different stearoptene contents.

Chapter IV Essential Oil from the Seeds and Carpels of Zanthoxylum limonella Part I Identification of Components and Isolation of a new sesquiterpene

Seeds and carpels of Zanthoxylum limonella on steam distillation yielded 6·1% of a pleasant smelling oil The oil on chemical examination was found to be composed of 1-s abinene 35 8%, 1-α-pinene 17 9% carene 15·9% β-pinene 12·2% α-thujene 4·8% terpinen-4-ol 2·9% dihydrocarvool 1 1%, dihydro-α terpineol 0 7% α-caryophyllene 1 3% and a new sesquiterpene (provisionally named as limonellene) 0·9%

Chapter V Essential Oil from the Seeds and Carpels of Zanthoxylum limonella Part II Studies on the Constitution of Limonellene

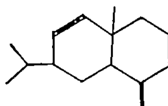
The new hydrocarbon (Limonellene) has been isolated from the higher boiling fractions by chromatography It possessed the following properties

$$b_{15}\ 135\ C \quad d^{30}_{30}\ 0{\cdot}8896. \quad n^{30}_{D}\ 1{\cdot}490 \quad \alpha_{D}\ 12{\cdot}0^{\circ}$$

The hydrocarbon was found to be bicyclic The two double bonds are unconjugated and one of them on the basis of infrared spectra appeared to be a terminal methylene group

On dehydrogenation with selenium eudalene was formed. On ozonolysis formaldehyde was obtained as a volatile product. Acetone was, however absent in the products of ozonization. The non-volatile residue obtained after ozonolysis was found to give a positive test for aldehyde and negative test for methyl-keto-group The non volatile residue on further oxidation with potassium permanganate gave a dibasic acid. It was also observed that neither the non-volatile produ t of ozonolysis of limonellen nor the oxidation product of the above gave any colour reaction with ferric chloride

On the basis of the above observations, the following tentative structure of limonellene has been proposed.



Chapter VI Essential Oil from the Peels f Citrus Microcarpa Bunge

Fresh peels of Citrus microcarpa on steam distillation gave 0 71% of a pleasant smelling essential oil. It was studied in detail and the following components were identified.

d-limonene and dipentene 91 85 %, linalool 2·47%, α-terpineol 1 30%, linalyl acetate 1 37%, citral 1·06 % geraniol 0·58 % methyl anthranilate 0·15% and citroptene 0·31%

Chapter VII Essential Oil from the Flowers of Melia azadirachta (Neem) Part I Identification of Components and Isolation of New Sesquiterpenes

The essential oil of Melia azadirachta flowers was obtained in a yield of 0 025% and was found to possess the following components on detailed chemical examination

Thio-amyl alcohol 7·6% benzyl alcohol 9·67% benzyl acetate 8·2%, an unidentified alcohol 3·9% and three new sesquiterpenes. The new sesquiterpene hydrocarbon had the following physical characteristics

Particulars	*No I*	*No II*	*No. III*
Percentage	8 7	15 7	30·8
Boiling point	132°C/12 mm	134°C/12 mm	135°C/9 mm.
d^{30}_{30}	0 9012	0·9038	0·9079
n^{30}_{D}	1 892	1 496	1·502
αD	19 5	11·0	-2·5

Chapter VIII Essential Oil from the Flowers of Melia azadirachta (Neem) Part II Studies on the Constitution of New Sesquiterpenes.

Three new sesquiterpene hydrocarbons were isolated from the higher boiling fractions of the oil. The first and the third were named as Azadirachtene and Margosene respectively

Azadirachtene ($C_{15} H_{24}$)

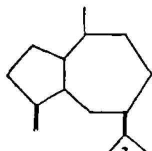
It was found to have the following characteristics —

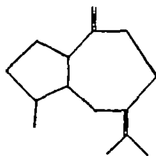
b_{12}132°C, d^{30}_{30} 0·9012., n^{30}_{D} 1 492., α_D 19·5 Mol. Ref. 65·9 No. of double bonds 2

The hydrocarbon was found to be bicyclic in nature. The two double bonds were found to be unconjugated and one of them on the basis of infrared spectrum appeared to be a part of terminal methylene group

On dehydrogenation with selenium, S-guiazulene was formed. Ozonolysis of the hydrocarbon gave a negative test for aldehyde and methyl-keto group

On the basis of these observations azadirachtene appears to possess one of the following structures —





The Second Hydrocarbon (C_{15} H_{24})

The hydrocarbon had the following characteristics

b_{12} 134°C. d^{30}_{30} 0·9038., n^{30}_{D} 1·492 α_D −11·0., Mol. Ref 66 1., No of double bonds 2·08.

The hydrocarbon appeared to be bicyclic as evident from the value of molecular refraction and number of double bonds. The two double bonds were unconjugated and one of them was present in the terminal methylene group On dehydrogenation with selenium S-guiazulene was formed

Margosene ($C_{15}H_{24}$)

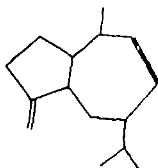
The hydrocarbon was found to have the following physical characteristics —

b_9 153°C., d^{30}_{30} 0·9079 n^{30}_{D} 1 502., α_D 2., No of double bonds 2 12.

The hydrocarbon is bicyclic in nature gives and S-guaiazulene on dehydrogenation with selenium. The two double bonds present in the hydrocarbon were unconjugated and one of them was a part of terminal methylene group, as evident from infrared studies.

Ozonolysis of the hydrocarbon gave formaldehyde as a volatile product. Acetone was absent in the products of ozonization The non-volatile residue after ozonolysis gave a positive test fo aldehyde group and a negative test for methyl-keto group The ozonised product formed a dibasic acid on oxidation with potassium permanganate. It also showed a negative response to ferric chloride showing the absence of enolisable hydrogen in the product of ozonolysis

On the basis of observations the following tentative structure of margosene was proposed



Chapter IX Essential Oil from the Flowers of Michelia champaca Part I Identification of components and Isolation of a New Sesquiterpene Champacene

Flowers of Michelia champaca yielded 0 0;6% of a pleasant smelling essential oil. The oil on a detailed chemical examination was found to possess chiefly a new sesquiterpene hydrocarbon isolated in a yield of 47 3% in pure form. Studies on th sesquiterpene revealed it to be bicyclic in nature The sesquiterpene hydrocarbon was named as Champacene.

The minor components in the oil were as follows

1.8 cineole 9 9% a terpinene 1 7 % a terpineol 8·0% phenyl ethyl alcohol 2·9%., linalool 1·4% citral 1·2% and terpinyl acetate 0·6%.

Chapter X Essential Oil from the Flowers of Michelia champaca Part II Studies on the Constitution of Champacene

The new sesquiterpene hydrocarbon 'Champacene isolated from the higher boiling point fractions after being purified by chromatography was found to possess the following physical characteristics —

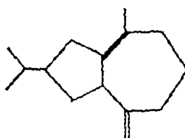
$$b_{8\,5}\ 117\ C.\quad d^{25}_{25}\ 0\text{-}8768\quad n^{25}_{D}\ 1\text{-}4915\quad a_D\ 12\ 6\text{-}$$

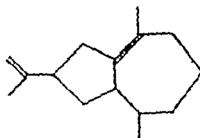
The hydrocarbon was found to possess two unconjugated double bonds. One of them appeared to be a terminal methylene group as evident from infrared spectra and the results of ozonolysis

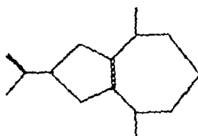
Dehydrogenation of Champacene with selenium gave vetivazulene. On ozonolysis formaldel yde was formed Acetone could not be identified in the volatile matter The non-volatile residue obtained after ozonolysis gave a negative test for aldehyde group and positive test for methyl-keto group. The non-volatile residue could not give any caboxylic acid on further oxid-

tion with potassium permanganate. The non volatile residue gave a 2 4 dinitro phenyl hydrazone which on repeated crystallisation with alcohol gave a sharp mp 113-114° C. Semi-micro combustion analysis of the non volatile residue and the corresponding 2 4 dinitrophenyl hydrazone suggested their molecular formulae as $C_{11}H_{22}O_{3}$ and $C_{23}H_{34}N_{11}O_{12}$ respectively

On the basis of these observations Champacene appeared to possess one of the following three structures







CHEMICAL EXAMINATION OF SYNTHETIC MIXTURES AND NATURAL ESSENTIAL OILS

RAM NAGINA LAL†
Chemical Engineering & Technology Section
Harcourt Butler Technological Institute Kanpur

In addition to the introduction in which literature on the related problem has been reviewed the subject matter of the thesis entitled Chemical Examination of Synthetic Mixtures and Natural Essential Oils has been divided into eleven chapters. The first chapter deals with the derivation of formulae for the estimation of the constituents of complex synthetic mixtures and fractions of an essential oil. The six chapters incorporate the studies of various formulae on several synthetic mixtures so as to establish the general applicability of those formulae. The two chapters deal with the application of formulae to the fractions of ternary and quaternary mixtures with a view to find out whether those formulae are applicable for the estimation of the constituents of such ternary and quaternary mixtures or not. The last two chapters record the investigations on the constituents and the determination of the per cent composition of spear mint oil and palmarosa oil by using formulae based on the physico-chemical properties of the fractions of the oils and their components.

In the course of present investigations, about eighty synthetic mixtures of different systems have been analysed by the application of various physico-chemical formulae and acetylation method.

Twentyseven synthetic mixtures of different compositions of the following binary systems have been analysed by using physico-chemical formulae 1 2 in chapter II

1 Geraniol and Acetophenone system
2. Benzyl alcohol and Acetophenone system
3. Phenyl ethyl acetate and Benzyl acetate system
4 Benzyl butyrate and Benzyl acetate system
5 Santalol and Geraniol system
6 Dimethyl benzyl carbinol and Terpineol system
7 Phenyl ethyl alcohol and Carvone system

$$a = 100\,(h_m - h_b)/(h_a - h_b) \quad (1)$$
$$b = 100\,(h_m - h_a)/(h_b - h_a) \quad (2)$$

where a and b represent the percentage of two components A and B of the mixture. Further h_a, h_b and h_m stand for the specific volumes of components A, B and the mixture respectively

This is an abstract of the thesis submitted for the Degree of Doctor of Philosophy Agra University Agra.

† Present address C/O Prof Ramji Sahai, Lakdirabad, Darbhanga (Bihar)

From the results of the above systems, the conclusion has been drawn that the formulae are suitable for the estimation of constituents of binary mixtures or fractions of an essential oil.

In chapter III, eight synthetic mixtures of different compositions of the following ternary systems consisting of two alcohols and one ester have been analysed using formulae 3 4 and 5 based on the specific volumes of the mixture and its components.

1 Benzyl alcohol, Phenyl ethyl alcohol and Benzyl acetate system

2. Phenyl ethyl alcohol, Phenyl propyl alcohol and Benzyl acetate system.

$$a = \frac{100(h_m - h_b) - c(h_c - h_b)}{(h_a - h_b)} \quad \ldots \quad \ldots (3)$$

$$b = \frac{100(h_m - h_a) - c(h_b - h_c)}{(h_b - h\ )} \quad \ldots \quad (4)$$

$$c = \frac{100v}{V} \quad \ldots \quad (5)$$

where a, b and c are the percentages of alcohols A, B and ester C having respective specific volumes h_a, h_b and h_c. Further h_m stand for the specific volume of mixture, V_e and v_1 are the ester values of pure ester C and of the mixture before acetylation respectively

From the results obtained it has been established that the formulae are suitable for the determination of the constituents of the synthetic mixture when the difference in densities of the constituents of the mixture is not less than 0·015 In addition similarity in chemical nature of the components on which depends the ideal mixture behaviour also play an important role in yielding better results.

In chapter IV fourteen synthetic mixtures of ternary systems consisting of two esters and one alcohol have been analysed using formulae 6, 7 and 8. The results prove that these formulae are applicable for determination of the percentage composition of such mixtures or fractions consisting of two esters and one alcohol of an essential oil although the results deviate slightly from the actual percentage of the constituents present.

1 Linalyl acetate, Santalyl acetate and Geraniol system

2. Benzyl acetate, Phenyl ethyl acetate and Geraniol system

3 Benzyl acetate, Phenyl ethyl acetate and Phenyl ethyl alcohol system

$$b = \frac{100\left[\left(1 - \frac{v_1 M_e}{56104}\right)(M_d h_d - M_e h_e) - \left(h_m - \frac{v_1 M\ h_e}{56104}\right)(M_d - M_e)\right]}{[M_d(h_d - h_b) - M\ (h_e - h_b)]}$$

$$c = \frac{M\left[100\,h_m - bh_b - \frac{v_1 M_d h_d}{561 \cdot 04}\right]}{[M_c h_c - M_d h_d]} \tag{7}$$

$$d = \frac{M_d\left[100\,h_m - bh_b - \frac{v_1 M_c h_c}{561.04}\right]}{[M_d h_d - M\,h_c]} \tag{8}$$

where b, c and d stand for the percentage of esters B, C and alcohol D having respective molecular weights M_b M and M_d. Further h_b h_c h_d and h_m represent the specific volumes of components B C, D and the mixture respectively while v_1 represents the ester value of the mixture before acetylation.

From these investigations the conclusion has been drawn that if one of the esters present in such ternary mixture is an ester of alcohol present in the mixture that is the constituents form a more ideal mixture better agreement with the theoretical percentage composition is obtained inspite of small difference in densities of the individual components.

In chapter V the analysis of a ternary mixture consisting of three alcohols namely benzyl alcohol, phenyl ethyl alcohol and phenyl propyl alcohol and a quaternary mixture consisting of the above three alcohols and benzyl acetate has been made by the physico-chemical methods and chapter IX incorporates the analysis of these mixtures by the acetylation method. In these cases the formulae have not been directly applied to the mixtures but to their fractions

In the case of the ternary mixture, the fractions have been assumed to contain only two components and thus physico-chemical formulae 1,2 previously discussed were applied to the fractions so as to determine their percentage composition and then of the mixture as a whole Similarly the analysis of the quaternary mixture was made by the application of physico-chemical formulae 3 4 and 5 described previously

The analysis of these mixtures has been made by the acetylation method using formulae 9 10 and 11 given below —

$$a = \frac{M}{M_a - M_b}\left[100 - c - \frac{M_b(v_2 - v_1)}{561 \cdot 04 - 0 \cdot 420 1 v_2}\right] \tag{9}$$

$$b = \frac{M_b}{M_b - M_a}\left[100 - c - \frac{M\,(v_2 - v_1)}{561\,04 - 0 \cdot 420 1 v_2}\right] \tag{10}$$

$$c = \frac{100 v_1}{V} \tag{11}$$

where a and b stand for the percentage of alcohols A and B having respective molecular weights M and M_b in the fractions of the ternary as

well as quaternary mixtures while c represents the percentage, in the quaternary mixture, of ester G having molecular weight M_4. Further v_1, v_2 and V_4 are the ester values of a fraction before and after acetylation and of pure ester G respectively

The results obtained for the ternary and quaternary mixtures indicate that the formulae are applicable to the close cut fractions of a complex synthetic mixture for finding out the percentage composition of the fractions of the mixture and subsequently of the mixture as a whole High reflux ratio during the course of fractionation of the mixture is recommended so that fractions consisting of two alcohols or two alcohols and one ester may be obtained

In chapter VI a comparative study of the usual method and the modified method for determination of the saponification value during the course of the determination of the percentage of the constituents of the mixture has been made with fifteen mixtures of the following three systems —

1 Benzyl acetate and Phenyl ethyl alcohol system,

2 Benzyl acetate, Phenyl ethyl acetate and Geraniol system,

3 Phenyl ethyl alcohol Phenyl propyl alcohol and Phenyl ethyl acetate system

In the case of system 1 the percentage of alcohol and ester was determined with the help of the ester value of the mixture before and after acetylation whereas formulae 12 and 13 have been used for the determination of the constituents of system 2

$$a = \frac{100v_1 - (100-c)V_b}{V - V_b} \qquad \ldots (12)$$

$$b = \frac{100v_1 - (100-c)V}{V_b - V} \qquad \ldots (13)$$

where a b and c stand for the percentage of esters A, B and alcohol C while v_1 V and V_b represent the ester values of the mixture before acetylation and of pure esters A and B respectively

Further formulae 9 10 and 11 previously mentioned have been applied for the determination of the constituents of system 3.

From the comparison of the results obtained by the usual and modified methods the definite conclusion has been arrived at that the percentage of the individual constituents of a synthetic mixture consisting of various constituents is more [illegible] determined by the application of various formulae derived using modified procedure of determination of saponification value

Chapter VII incorporates the analysis of eight synthetic mixtures belonging to ternary and quaternary systems as given below by modified technique of analysis

1 Linalyl acetate Santalyl acetate and Geraniol system,

2 Phenyl ethyl alcohol Phenyl propyl alcohol, Phenyl ethyl acetate and Lauric acid system.

For the analysis of system 1 formulae 12 and 13 and for system 2 formulae 9 10 and 11 previously discussed have been used. The conclusion has been drawn that the modified procedure for the determination of saponification value which gives ester value correct upto one decimal place results in more precise determination of the per cent of the individual constituents present.

In chapter VIII four synthetic mixtures of a ternary system consisting of benzyl alcohol phenyl ethyl alcohol and benzyl butyrate have been studied by the application of formulae 9 10 and 11 and it has been found that the observed percentage of the two individual alcohols differ considerably from the actual composition Thus a definite indication regarding the interference in the estimation of alcohols due to the presence of benzyl butyrate an ester other than acetate has been obtained, whereas the same formulae when apply to a similar ternary system consisting of phenyl ethyl alcohol, phenyl propyl alcohol and phenyl ethyl acetate previously discussed give excellent results.

In chapters X and XI investigations on constituents and the determination of the percentage composition of spearmint oil and palmarosa oil have been incorporated. In the case of spearmint oil formulae 14 15 were applied to the decarvonised spearmint oil as well as to its close cut fractions so as to determine the percentage composition of the decarvonised spearmint oil directly as well as for the per cent composition of its fractions.

$$c = \frac{100(h_d - h_m) - a(h_d - h_a) - b(h_d - h_b)}{(h_d - h_c)} \qquad (14)$$

$$d = \frac{100(h_c - h_m) - a(h_c - h_a) - b(h_c - h_b)}{(h_c - h_d)} \qquad (15)$$

In the above formulae a, b, c and d stand for the percentage of alcohol A, ester B and hydrocarbons C and D respectively while h_a h_b h_c, h_d and h_m stand for the respective specific volumes of the componets A, B C, D and the mixture.

For the percentage composition of the decarvonised oil, more or less the same result is obtained whether it is derived by application of the formulae directly to the decarvonised spearmint oil itself or to its fractions. From the percentage composition of the decarvonised spearmint oil the percentage composition of spearmint oil was determined.

So far the investigations on palmarosa oil are concerned, two sets of experiments on the same oil were performed. First, the oil was fractionated under reduced pressure maintaining high reflux ratio and the percentage composition of each fraction obtained from both the lots of palmarosa oil was determined using formulae 3 4 and 5 given previously as the fractions were assumed to have only three components. The results obtained from both the sets of experiments are more or less the same.

It has been observed that with the help of simple apparatus and different sets of formulae it has been possible to determine the individual percentage of components present either in mixtures or essential oils. Thus the various new formulae for the determination of the various constituents of a mixture consisting of a few perfumery compounds constitute a new tool in the hands of essential oil chemists for the determination of the constituents of a synthetic mixture or fractions of an essential oil and subsequently of essential oil itself.

STUDIES IN PARACHOR*

V G Vaidya

Chemistry Department, Holkar College Indore †

Summary

The investigation of complex formation and hydrogen bonding by finding out the parachor of solutions was undertaken by me for the present work. The work was divided into three sections —

(i) Closer examination of the application of the Mixture Law of Hammick and Andrew

(ii) Detection of complex formation by Job's method

(iii) Structure of inorganic oxy-acids such as nitric sulphuric, orthophosphoric and boric acids by the study of their parachor in pure state and in solutions. The results obtained and the conclusions drawn therefrom are as follows

(1) *Parachors in Solution*

The closer examination, I made, of the mixture law has revealed certain facts and certain conclusions have been drawn as to the conditions under which it can be applied and how best it can be applied.

Following conditions have been established —

(i) that in testing the mixture law P_m should be plotted against x. Since the equation $P_m = xP_x + (1-x)P$ on rearranging as $P_m = x(P_x - P_s) + P_s$ takes the form of a straight line ($y = mx + c$) there is no point in plotting P_x vs x

(ii) that application of x^2 test revealed that the law is sound and can be applied safely

(iii) that the value of the parachor of pure solute be calculated not by extrapolation of the straight line obtained by plotting P_m vs x but by analysing the experimental data by the least square method of curve fitting. I have applied this method in the case of boric acid

(iv) that the straight line mixture law is applicable provided that

- (i) there is no ionisation,
- (ii) there is no hydrogen bonding
- (iii) there is no complex formation and that,

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* Summary of thesis submitted to and approved by the Agra University Agra for the Degree of Doctor of Philosophy

† Present address—Govt. College Shahdol (M P)

(iv) the solubility of the substance under investigation is sufficiently high so that the error in calculating Px from the equation $Pr = x.Px + (1-x)\ Ps$ is not unduly magnified.

(2) *Parachor in detection of complex formation*

Job's method of continuous variation was used by me for studying complex formation between glycine and cobalt-nitrate, glycine and lead nitrate, formation of the dichromate ion and the complex formation between iodine and benzene and iodine and ethyl alcohol. Following results were obtained :—

(i) *Lead Nitrate and Glycine*

I have found that the complex ion PbG^+ exists in solution, i. e., complex formation occurs at the volume ratio of 1 : 1. Monk has studied the system by pH titrations and found two complexes PbG^+ and PbG_2. Fuseya and Murata from migration studies and Karmalkar and Bafna from polarographic potentiometric and conductometric studies report only one, i. e. PbG^+. These results confirm my observations.

(ii) *Cobalt Nitrate and Glycine*

Monk has reported two complexes CoG^+ and CoG_2 in this system from his pH titration studies. I have studied the system at two different concentration—M/5 and M/10—solutions. In the former I found CoG^+ and CoG_2 complexes whereas in the latter only one CoG^+. The absence of CoG_2 in M/10 solutions may be due to its greater instability.

(iii) *Formation of dichromate ion*

Vosburgh and Cooper studied the formation of dichromate ion spectrophotometrically using Job's method of continuous variation. A similar study was undertaken by parachor measurements. Ostwald (Z. physikal. Chem. 1888 2,78) and Abegg and Cox (Z. physikal Chem. 1904 18, 510) and also Datta and Dhar (J. Am. Chem. Soc. 1916, 38 1303) carried out the physico-chemical investigations dealing with aqueous solutions of Chromic acid. Using M/10 solutions of potassium chromate and hydrochloric acid, I found that the dichromate ion is formed when the solutions are mixed in the ratio of 1 : 1.

(iv) *Complexes of iodine with benzene and ethyl alcohol*

Iodine forms brown solutions with certain organic solvents. An examination of the brown solutions by Beckman and Stock, Grob, Getman, and others have proved that iodine is chemically bound in these solutions. I have investigated the nature of complexes of iodine with benzene and ethyl alcohol. I found that $C_2H_5OH\ I_2$ and C_6H_6-I_2 are the complexes formed. Hilderbrand and Benesi from spectrophotometric studies found that iodine and

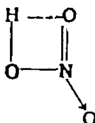
benzene form the complex $C_6H_6 I_2$. Enzo Ferroni and Gabriela Gabrielli from surface tension-measurements concluded that the complex $C_2H_5OH I_2$ is formed between iodine and ethly alcohol These results are in confirmity with my observations

(3) *Hydrogen Bonding and the structure of nitric sulphuric ortho-phosphoric and boric acids*

Parachor studies of pure acids and their solutions in water were made with a view to understand their molecular structure. Following results were obtained. It was shown that a parachor decrease upto 12 units corresponds to a hydrogen bond (Bhagwat and Shukla)

(i) *Nitric Acid*

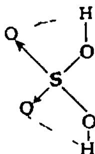
The observed parachor of pure nitric acid was found to be about 9 units less than the calculated value. This difference was explained by the presence of one intramolecular hydrogen bond. The structure of nitric acid can be



Yost and Russel have suggested a similar structure but no definite proof was given by them

(ii) *Sulphuric Acid*

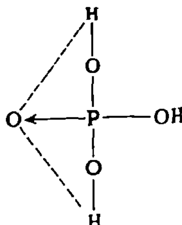
The difference in calculated and observed parachor value of sulphuric acid is about 16 units. This can also be explained by assuming that there are two intramolecular hydrogen bonds.



Venkateswaran has suggested hydrogen bonded structure for sulphuric acid from Raman spectra studies.

(iii) *Ortho-phosphoric acid*

An anomaly of about 12 units exists between the calculated and observed values of parachor of orthophosphoric acid. The presence of two intramolecular hydrogen bonds can explain the anomaly. The structure proposed by me is



Venkateswaran from Raman spectra studies of di-sodium-hydrogen phosphate has found the presence of hydrogen bonds. Hendricks and West have detected hydrogen bonds in potassium dihydrogen phosphate from crystallographic studies. These results support my conclusions

(iv) *Boric Acid*

The observed and calculated values of parachor of boric acid differ by about 7 units. Pauling from crystal studies states that there are hydrogen bonds in boric acid. Wells has also detected hydrogen bonds but he calls them as hydroxyl bonds and states that these bonds are weaker than the hydrogen bonds. I Kabovec from Raman spectra examination states that these should be some kind of hydrogen bonds. This difference of 7 units in observed and calculated values of parachor can be due to hydroxyl bonds which are weaker

The low solubility of boric acid in water gave some difficulty in calculating its parachor from the parachor of its solutions. This was overcome by applying the method of least squares. The equation to the best fitting curve was found out and putting the value of $x=1$ in it gave the value of the parachor of pure boric acid.

STUDIES ON THE MORPHOLOGY VIABILITY AND PRESERVATION OF POLLEN GRAINS OF MANGO (*MANGIFERA INDICA* L.) LITCHI (*LITCHI CHINENSIS* Sonn.) AND LOQUAT (*ERIOBOTRYA JAPONICA* Lindl.)*

S N SINGH†

Fruit Breeder Govt Horticultural Research Institute Saharanpur

SUMMARY

The pollen grains are the carriers of hereditory characters and play a vital role in the evolution of new varieties. They are also important in taxonomy since the characteristic variations in pollen morphology aid in the classification of various genera and families of the plant kingdom. The review of available literature has shown that while a good deal of work on the various aspects of the pollen grains of temperate fruits is on record, similar information in respect of tropical and sub-tropical fruits, particularly mango litchi and loquat is rather scanty. With this objective in view the present studies on the morphology viability and preservation of the pollen grains of mango litchi and loquat were undertaken at the Horticultural Research Institute, Saharanpur during 1958-60 and the results are summarized below —

1 Studies on the pollen morphology of 50 varieties of mango, 10 of litchi and 12 of loquat showed similarity in symmetry aperture, shape and size. The grains were isopolar radiosymmetric, angulaperturate and 3-colporate. Unlike litchi, the sexine in mango was slightly thicker than nexine, while in loquat it was as thick as nexine. The sexine pattern in mango was reticulate and in litchi and loquat, it was granulate. The shapes of the pollen of mango, litchi and loquat were sub-prolate, sub-prolate to prolate, and prolate, respectively. In size, the pollen of litchi (19·9 to 23·5 microns) and loquat (23·3 to 25 microns) belonged to the small spores class, whereas in mango the grains of 29 varieties (25·1 to 28·3 microns) belonged to the medium spores class and of the remaining 21 varieties (23·5 to 24·9 microns) to the small spores class.

In all the three fruits, the pollen were 2-celled at the time of shedding. The mitotic division leading to the formation of the vegetative and generative cells was clearly observed in the pollen grains of mango, varieties Baramasi, Safeda Malihabad, Fajari Gola and Rataul.

2. Occasionally some pollen grains of 'giant' size were observed in the varieties, Alphan and Asanjia Surkha in mango, Khatta in litchi and Improved Golden Yellow and Improved Pale Yellow in loquat, showing 4 germpores instead of 3 suggesting the formation of restitution nuclei with double the number of chromosomes.

* Summary of the Thesis submitted to and approved by the Agra University Agra for the Degree of Doctor of Philosophy in Horticulture.

† Present address : Asstt. Prof. of Horticulture Government Agricultural College, Kanpur.

3 The examination of the pollen grains in aceto-carmine exhibited mean pollen sterility of 1·02 to 6 51 per cent in mango, indicating a negligible variation. In litchi and loquat, the percentage of imperfect grains was comparatively higher and ranged from 8 04 to 48 39 per cent and 7·56 to 26 31 per cent, respectively Among the litchi varieties, Early Seedless (46·1 to 48 39%) and Khatti (42·68 to 44·03%) and in loquat, varieties California Advance (24 32 to 26 31 %) and Improved Golden Yellow (24 2 to 25·03%) exhibited high percentage of defective pollen. The pollen sterility appeared to be due to the degeneration in the formation of the microspore or due to hybridity

4 The study of the atmospheric pollen revealed almost similar trend in the case of mango litchi and loquat, and indicated that their pollen are not air borne, thus showing the limitations of anemophilous pollinations in these fruits.

5 (a) The results of the experiments on pollen germination in mango in artificial media showed that the highest mean germination of 37 75 per cent and tube length of 122 84 microns were obtained in 25 per cent sucrose solution in the variety Samar Bahisht Alibagh after 24 hours of incubation at 25°C, while the variety Bombay Green gave the mean pollen viability of 39·69 per cent and tube length of 141 79 microns after 18 hours of incubation. The results obtained in 25 per cent sucrose solution were significantly superior to the remaining concentrations excepting 26 and 24 per cent strengths. Concentrations of 35 and 15 per cent gave the lowest pollen germination with no significant difference between them. There was however no pollen germination in 0 5 10 and 40 per cent sucrose solutions in repeated tests. In exceptional cases, high pollen germination of 44 57 and 52 18 per cent was observed on two dates in 25 per cent, sucrose solution.

Germination tests with the pollen of 10 varieties of mango showed a varietal response. The varieties Samar Bahisht Alibagh, Bombay Green and Bombay Yellow exhibited the highest mean viability of 37 56 to 40·78 per cent and the varieties Romani and Rataul gave the lowest mean viability of 15 57 to 16 37 per cent with no significant difference between them.

(b) In the case of litchi pollen, the highest mean germination of 62·62 and 61 13 per cent was achieved in 15 per cent sucrose solution after 4 hours of incubation in the varieties Rose Scented and Calcuttia, respectively and the results were significantly superior to the other concentrations. 35 per cent sucrose medium gave the lowest mean viability of 7 53 to 9·42 per cent. Similarly sucrose concentration of 30 1 and 0 per cent gave a low viability with no significant difference between them. In another experiment, the maximum mean germination of 63 42 per cent and tube length of 366 41 microns were obtained after 4 hours of pollen planting at 30°C. Thereafter there was no further increase in viability although the pollen tubes continued to elongate up to 24 hours at 30°C and attained the length of 971 75 microns

Of the 10 varieties tested for pollen viability Rose Scented, Dehradun Late Seedless, Calcuttia and Pickling exhibited the maximum mean pollen germination of 59·61 to 61·99 per cent, with no significant difference between them, while Early Seedless showed the lowest mean viability of 36·22 per cent only Khatti and Gulabi varieties also gave a low germinability of 42 52 and 45·87 per cent, respectively Besides, the pollen collected from the staminate and pseudo-hermaphrodite flowers exhibited viability of 61 54 and 48 53 per cent, respectively whereas the pollen from hermaphrodite flowers indicated the lowest mean germination of 7 8 per cent. The anthers of the bisexual flowers did not dehisce and hence their pollen was extracted by needle after anthesis.

(c) Germination trials conducted with the pollen of loquat variety Golden Yellow showed that 1 2 5 and 5 per cent sucrose concentrations gave the maximum mean viability of 79·14 to 79·9? per cent after 2 hours of incubation at 30°C 35 per cent sucrose solutions exhibited the lowest germination. Of the different varieties, the pollen of Pale Yellow gave the highest mean viability of 82·95 to 83·61 per cent in 1 to 5 per cent sucrose solutions while California Advance showed significantly lower germination of 73 81 to 75·2 per cent. The treatments—sugars, varieties and their interactions indicated significant differences. The viability of loquat varieties tested at different periods of blossoming showed that pollen germination percentage and tube length were very low in the early and late blooms and high during the mid bloom. It seems probable that the non-setting in flowers during early and very late blooms is due to the lack of viable pollen.

6 (a) The pollen germination tests in sucrose-agar medium in mango variety Samar Bahisht Alibagh indicated that the maximum viability of 40·8 per cent and tube length of 175 3 microns were obtained in 25 per cent sucrose solution containing 0 5 per cent agar after 24 hours of incubation at 28°C, while the variety Bombay Green showed pollen germination of 41 59 per cent and tube length of 160·29 microns. Though th addition of 0·5 per cent agar enhanced pollen germination but it did not differ significantly from the control

(b) In litchi varieties Calcuttia and Rose Scented the highest mean pollen germination of 66·11 to 69·32 per cent and tube length of 404 15 to 409·63 microns were obtained in 15 per cent sucrose solution containing 1 per cent agar which were significantly superior to the other treatments. The next higher germination was observed in 15 per cent sucrose medium containing 0·5 per cent agar Among the agar levels, 1 per cent solution proved significantly better than the other concentrations, while in the sugar levels, there was no significant difference between 10 and 20 per cent concentrations.

(c) The germination of the pollen of loquat varieties Golden Yellow and Pale Yellow in sugar agar medium showed significant differences for the

sugar and agar levels In the sugar levels 1 per cent concentration gave the best results, but it did not differ significantly from 2·5 and 5 per cent sucrose solutions. However all these concentrations produced higher pollen germination than 7·5 and 10 per cent strengths. Of the agar levels, 0·5 per cent concentration gave the best performance and was significantly superior to the rest.

7 In the lactose medium, the mango pollen did not germinate, while the loquat pollen showed the highest mean viability of 66·28 to 66·85 per cent in 1 to 5 per cent concentrations. The pollen of 3 varieties of litchi, however, gave the maximum viability of 32·77 to 53·74 per cent in 15 per cent lactose solution.

8. The pollen of litchi exhibited the highest mean germination of 47·71 per cent in 15 per cent glucose medium though it did not differ significantly from 20 per cent concentration In loquat, however 5 per cent glucose solution showed the highest average pollen germination of 64·45 per cent, but it did not differ significantly from 10 and 15 per cent strengths.

9 (a) Of all the plant regulators and other pollen stimulants used with mango pollen 20 ppm boric acid and borax gave the highest pollen germination, which were closely followed by 10 ppm IBA and then by 20 ppm NAA 10 and 30 ppm concentrations of IBA and NAA gave lower viability than the control. All the concentrations of the remaining plant regulators were ineffective and proved rather toxic.

(b) In litchi 20ppm boric acid and borax produced the maximum pollen germination and tube length, which were significantly superior to the rest. Concentrations of 10 ppm of IAA, 20 ppm of IBA, NAA, 2,4-D and 30 ppm of IPA and gibberellic acid gave higher pollen germination and tube length than the control. The length of the pollen tubes exhibited marked increase in boric acid and borax treatments. 2 4 5-T and colchicine were ineffective as compared to the control, due to toxicity

(c) In loquat, 10 ppm boric acid, borax and IAA gave the maximum pollen germination and tube length with no significant difference between them. Besides, 10 ppm NAA, 20 ppm IBA, 2 4-D gibberellic acid and IPA also exhibited higher pollen germination than the control. Similar to mango and litchi, all concentrations of 2 4 5-T and colchicine produced inhibitory effect on pollen viability in loquat.

10 The dry pollen of loquat incubated for 24 hours in a moist petri dish at 30°C, gave the mean viability of 23·83 per cent and pollen tube length of 87·68 microns by absorbing moisture. It indicated that the pollen in the flowers get readily spoiled in rains and on foggy days during winter or thereby reduce the fruit-set.

11 The observations on the open pollinated pistils in mango showed that 2·5 to 35 per cent stigmas received the pollen and thus indicated that in nature over 65 per cent bisexual flowers remain unpollinated, whereas fairly high percentage of pistils in litchi are pollinated In loquat, the carpels collected in early and late blooms exhibited that the lowest percentage of stigmas are pollinated in nature The L. S of carpels indicated that fertilization occurred in 48 hours in mango, and within 24 hours in litchi and loquat.

12. The study of growth rate of the pollen tubes showed that the maximum growth was obtained after 12 hours of incubation at 28°C in mango while it was the highest in litchi and loquat at 30°C, and continued to elongate up to 24 hours

13 (a) The pollen storage trials in mango indicated that at the room temperature, the pollen remained alive for 12 to 20 days in desiccators and for 8 days in the petri dish. At low temperatures, the pollen stored in desiccators exhibited the maximum longevity of 14 months under deep freeze (23°C) of 7 to 10 months at 0°C and of 5 months at 4 5° and 9°C. 0 per cent R. H. in the desiccators at 0°C and room temperature gave comparatively higher viability than other R. H conditions, and 50 per cent R. H did not favour the storage longevity due probably to high humidity

(b) The litchi pollen stored at the room temperature remained viable for 3 to 5 months in the desiccators and for 10 to 30 days in the petri dishes. It showed storage longevity of 31 and 15 months at 23 and 0°C, respectively The longevity was, however very low (9 to 12 months) at 9° and 4·5°C. The pollen sample of the variety Early Seedless gave comparatively much lower longevity than that of Calcutta at all the temperatures. Of the various R. H. conditions maintained at the room temperature and at 0°C, 25 and 50 per cent R. H. gave the lowest viability Among the pollen grains of varieties stored at 23°C and in petri dish at the room temperature, Rose Scented pollen gave the highest viability while Early Seedless showed the lowest germinability

(c) The storage trials with loquat pollen showed that at the room temperature they remained alive for 35 to 45 days in the petri dish and for months in the desiccators. The pollen preserved at the low temperature exhibited the highest longevity of 26 months at 23°C. The pollen remained viable for 23 months at 0°C, for 17 to 19 months at 4.5°C and for 12 to 16 months at 9°C. The variety Pale Yellow showed better storage viability than California Advance and Improved Golden Yellow 25 per cent R. H at the room temperature and 10 per cent R. H. at 0°C gave higher viability than the remaining R. H conditions.

14 The biological reactivity of the pollen of litchi and loquat was tested after 1 and 2 years of storage by field pollinations and subsequent fruit-set. Thus, encouraging results were obtained which showed that the stored pollen

sugar and agar levels. In the sugar levels 1 per cent concentration gave the best results, but it did not differ significantly from 2·5 and 5 per cent sucrose solutions. *However all these concentrations produced higher pollen* germination than 7·5 and 10 per cent strengths. Of the agar levels, 0·5 per cent concentration gave the best performance and was significantly superior to the rest.

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8. The pollen *of litchi exhibited the highest mean germination of 48·71* per cent in 15 per cent glucose medium though it did not differ significantly from 20 per cent concentration In loquat, however 5 per cent glucose solution showed the highest average pollen germination of 64·45 per cent, but it did not differ significantly from 10 and 15 per cent strengths.

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(b) In litchi, 20ppm boric acid and borax produced the maximum pollen germination and tube length, which were significantly superior to the rest. Concentrations of 10 ppm of IAA, 20 ppm of IBA, NAA, 2,4-D and 30 ppm of IPA and gibberellic acid gave higher pollen germination and tube length than the control The length of the pollen tubes exhibited marked increase in boric acid and borax treatments. 2, 4 5-T and colchicine were ineffective as compared to the control, due to toxicity

(c) In loquat, 10 ppm boric acid, borax and IAA gave the maximum pollen germination and tube length with no significant *difference* between them. Besides 10 ppm NAA, 20 ppm IBA, 2,4-D gibberellic acid and IPA also exhibited higher pollen germination than the control. *Similar to* mango and litchi, all concentrations of 2, 4 5-T and *colchicine produced* inhibitory effect on pollen viability in loquat.

10 The dry pollen of loquat incubated *for 24* hours in a moist petri dish at 30°C, gave the mean viability of 23·83 per cent and pollen tube length of 87·68 microns by absorbing moisture. *It indicated* that the pollen in the flowers get readily spoiled in rains and on foggy *days* during winter and thereby reduce the fruit-set.

INFLUENCE OF 2, 4-DICHLOROPHENOXY ACETIC ACID ON THE SEED QUALITY OF MAIZE[1]

S K. SINHA
D A V College Kanpur
AND
I M. RAO[2]
S V University Tirupati (A. P)

2, 4-Dichlorophenoxy acetic acid has been used as a weedicide by most of the workers but its property of beneficial effect on cereals has been elucidated only recently (Stromme and Hamner 1948 Bharadwaj and Rao 1955 56 Sinha 1957 Sinha and Rao 1961) During last few years influence of 2, 4-D on the metabolism has also been studied. Mitchell and Brown (1945) observed depletion in available carbohydrates (Sugars, dextrins and starch) in Morning Glory after the spraying of 1000 ppm of 2, 4-D In wild garlic Klingman and Ahlgren (1951) observed decrease in sugars and polysaccharides by the application of this chemical. In the present study an attempt was made to determine the percentage of available carbohydrates in seeds of maize obtained from the plants raised from the 2, 4-D treated seeds.

METHODS AND MATERIAL

Seeds of Maize T 41 were obtained from the Economic Botanist U P Govt. Kanpur and were given a pre-sowing soaking treatment of 24 hours in 0 10, 100 ppm of 2 4 D After the treatment period seeds were sown in pots filled with garden soil on 24th June 1956, along with the dry seeds as Control Three pots were alloted for each treatment and in each pot ten seeds were sown Thinning was done at fortnightly and monthly intervals and ultimately only three plants per pot were left for seed production Besides this Normal sowing a similar set was started on 9th July 1956 as Late sowing The pots were kept in a glasshouse.

The seeds obtained from this experiment were analysed for reducing sugars, invert sugars and starch by gravimetric method following Loomis and Shull (1937)

RESULTS

Observations summarising the percentage of sugars and starch in the Table are based on air dry weight of seeds.

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1 Part of the thesis submitted for the M. Sc. degree of Agra University 1957

2. Formerly Assoc. Professor of Botany Agra College Agra.

Reducing Sugars—The reducing sugars were observed only in the 100 ppm 2 4-D treatment of the Normal sowing These sugars were completely wanting in other treatments including the Control.

Invert sugars—Amount of invert sugars decreased with the supply of 2, 4-D It is interesting to note that no fluctuation occurred in the Controls of the two sowings but in 0 ppm amount of invert sugars decreased in the Late sowing The percentage of invert sugars in 10 ppm and 100 ppm increased in the Late sowing as compared to the Normal sowing.

Starch—By the application of 2 4 D the starch content of the seeds remained almost unchanged.

Discussion

In the present study an attempt was made to find out the changes in sugars and starch contents of the seeds obtained as a result of the 2, 4-D treatment. In both Normal and Late sowing percentage of invert sugars reduced to a little extent while in 100 ppm (Normal sowing only) reducing sugars were obtained. The percentage of starch remained almost unaffected except by 0 ppm and 100 ppm (Normal sowing) Thus it appears that 2, 4-D has no influence on the total available carbohydrates of the seeds. However Sinha (1957) observed beneficial effect of 2, 4-D in low concentration (0 1 and 1 ppm) on the seedling growth of maize. The higher concentrations of 10 and 100 ppm significantly inhibited the growth. Sinha and Rao (1961) reported enhanced rate of respiration of maize seedlings by 1 ppm of 2, 4 D and a decrease by 10 and 100 ppm. Thus it is evident that 2 4-D does influence various physiological processes but in the later stages the effect does not seem to continue.

Summary

The effect of pre-sowing soaking treatment of 2, 4-D on the available carbohydrates of the grains has been studied in maize. The results observed indicate that available carbohydrates are not affected by the 2, 4-D treatment.

Acknowledgements

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TABLE

Effect of 2 4-D on the quality of seeds of maize indicated by the percentage of sugars and starch.

Chemical analysis of seeds (%)	Normal Sowing Pre-soaking in 2 4-D				Late Sowing Pre-soaking in 2 4-D			
	Control	0 ppm	10 ppm	100 ppm	Control	0 ppm	10 ppm	100 ppm
Reducing sugars	—	—	—	7	—	—	—	—
Invert sugars	2 9	3 6	0 3	—	3 1	0 6	1 9	2 7
Starch	81 8	71 2	79 8	72 9	82 6	72 5	79 7	78 4

RHEOLOGY OF ADHESIVES*

R C. Gupta
Composite Wood Branch Forest Research Institute, Dehra Dun

Rheology plays an important role in adhesives and adhesion

In this thesis an attempt has been made to understand the part rheology plays in adhesives and adhesion.

The study therefore, was extensive and covered a wide range of adhesives and involved the development or adoption of suitable techniques for the study of the rheological properties in adhesive solutions, adhesives, films, processes taking place during gelling of adhesives and properties of set adhesives.

Viscosity plays an important role in adhesion and adhesives. If the adhesive is too thick it is difficult to spread If too thin it may penetrate (the permeability of the wood will also play an important role) and cause starved joints. Correct viscosity may help chain segments approach quite closely the interface. The correct thickness of the glue line will depend on the spread, viscosity and pressure.

In the present work the interesting observation that cold setting CNSL resin is dilatant was made The variation of viscosity with time with para formaldehyde content of over 8% may be described as follows —

$$n = n_o e^{kt}$$

The glue line thickness will be proportional to $\sqrt{n}$ and penetration will be governed by Poiseuile's law On plotting viscosity/time in log/log scale below 16% paraformaldehyde content the results are represented by two straight lines suggesting 'pregelation (mechanism suggested by Joly) With 16% no pregelation is noticed.

The time taken for the adhesive system to attain yield values and the yield values obtained run parallel to the findings of glue adhesion tests With thermosetting condensates it was noticed that the viscosity of 3×10^4 RWS was satisfactory with C. N S L. Xylol resin whereas with C. N S L. phenol, very much lower viscosity is required.

A study of the role of extractives has yielded valuable information With casein glues the role of extractives on viscosity confirmed the earlier indications of Handa and Sodhi. This was particularly striking with C N S L. resins where a close and striking parallelism between development of viscosity

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*This is summary of the thesis submitted to and approved by the Agra University Agra for the Degree of Doctor of Philosophy

yield value and glue adhesion strength was observed and thus the role of surfaces in adhesion amply demonstrated.

As not much data is available on the use of the Langmuir-Adam-Rideal technique in the study of adhesives, some preliminary investigations reported in the study indicate possibilities.

Membranometric measurements first suggested by Ostwald for the study of films on liquid surfaces have been applied for the first time to adhesives, and the results obtained in the present study clearly show the value of this method for the evaluation of adhesives, especially the development of tack.

It is necessary that adhesives when applied to the adherend surface spreads to form a continuous thin film. Adhesives are also available in film form. Therefore, a study of the properties of adhesives in film form formed an important part of the thesis.

The apparent modulus of rigidity of the film was found to increase with the load so that values extrapolated to zero load were used. With temperature the rigidity of gelatin films decreases to 60°C, remains constant and then a slight increase above 90°C. This suggested possible readjustment of structure in this region On the other hand with casein the interesting observation was made that the rigidity showed a continuous increase upto 100°C and then showed a fall. Prolamin showed a constant value upto 80°C and then fell rapidly almost flowing at 100°C. Examination of the apparent modulus without extrapolation to zero-load also indicated interesting results

As is to be expected moisture decreased the modulus evidently due to plasticisation Addition of 5% CNSL increased the modulus of rigidity of prolamin upto 20% R H Higher concentrations decreased the modulus at all humidities.

The method of preparation of the film had a marked effect possibly due to orientation effects, stress concentration or presence of air bubbles, etc

The tensile strength of the films varied with the material, the highest value being obtained with gelatin followed by casein the lowest value being that of prolamin

The product of the modulus of rigidity and log decrement was found to be constant as with wood.

A study of the creep properties of the film also indicated interesting results.

Studies on the influence of loading indicated plastic flow the effect being higher with casein Here also plasticisers increased the effect.

As the development of rigidity may be considered as an index of gel formation and strength, this was followed with interesting results covering a range of adhesives both natural and synthetic, viz protein (animal glue casein) resorcinol formaldehyde, urea formaldehyde, tannin-formaldehyde, and cashew nut shell liquid-formaldehyde resins

The curves for development of rigidity with time are similar fo (a) casein and tannin-formaldehyde and CNSL, (b) urea-formaldehyde and resorcinol-formaldehyde These for gelatin are different in shape. With gelatin there is no chemical transformation during setting whereas it was otherwise with the others The difference between adhesive setting contributed by chemical and physical mechanisms was also seen in the effect of temperature in that the development of rigidity and rigidity of gelatin decreased with temperature With Aerolite on the other hand the rate was found to double for every 5 degrees and also gave higher values with higher temperature

The development of rigidity with CNSL resins as influenced by para formaldehyde content confirmed the results obtained in the viscosity studies in that a minimum concentration of at least 12% is necessary there being a big jump from 14% to 16%

The lap joint tests with the glue also were found to run parallel with the rigidity values

The important role that wood extractives can play and which has not received sufficient attention so far was clearly brought out in these studies

Work done with gelatin gels is both of considerable theoretical and practical interest especially as India has an established animal glue industry

The development of rigidity in gelatin gels was intensively studied and the influence of various factors, viz. purity concentration, temperature and various additives including inorganic salts, acids, bases alcohol sugars hydrogen bond depressants, etc.

The kinetics of the development of rigidity was found to be highly concentration and temperature dependent. Being mainly a physical mechanism the rigidity falls with temperature. At 35°C at the highest concentration studied no gel formation takes place The rigidity/temperature curves for all concentrations studied converge to a minimum value at 35°C. The presence of an inflection point with two straight arms in the rigidity concentration curve noticed indicates a pregelation mechanism, i e the gel formation takes places in steps.

The work on the effect of ions, neutral molecules etc. done is of importance in the use of animal glue for adhesive work and for evolving suitable formulations.

Work on depressants carried out not only brought forth interesting results but enabled the development of thermosetting plywood glue formulae *thus making a valuable contribution* to the plywood industry

The gel formation mechanism of paddy husk extract was found to be similar to that of gelatin

That the strength and durability of glue *joints* will depend on the mechanical constants (elastic *moduli*) of the adherend and adhesive and their changes with temperature and psychrometric conditions is well recognized and theoretical studies have been made by Dietz *et. al* De Bruyne, Thread and others. In the moulding of plastics with thermosetting adhesives, stresses caused by the differences in thermal expansion can cause severe stresses and Turner has shown, based on stress equilibrium, equations can be set up to calculate the coefficient of thermal expansion based on the fraction per cent by weight, density and the bulk moduli (or Young's moduli) and thus suitable material added to the moulding powder With glued wood products the swelling and shrinkage stresses can be considerable. Dietz *et. al.*, have shown that stresses as high as 2000 lbs./sq in may be developed. In the long term loading of glued members creep characteristics are of great importance. The experiments carried out on this aspect therefore form a preliminary probe into this important aspect.

The strength (compressive) tests on the set adhesives indicated that C. N. S L. gels are weaker than those of Urea formaldehyde. *Many factors, e.g.* time, temperature, formaldehyde content, wood extractives, etc., were found to affect the final strength.

The results with C. N S L. resins indicate the possibility of pore formation with excess of formaldehyde or water vapour which may reduce the bond strength causing total failure a point hitherto not given due consideration.

The effect of extractives on the rate of setting, final strength and crazing of glue lines studied, is extremely important cases have come to light of plywood delaminating during storage after some months. Whether this is due to formation of a skin round the particles or other causes have to be studied.

The studies included the creep characteristics of C. N S. L. resins. The curves obtained are similar to those for perspex obtained by Marin (195? The curves can also be divided into three parts, the instantaneous elastic strain the transient creep strain and the steady state creep.

The relaxation studies carried out also gave interesting results and it would be useful to study creep and relaxation after different periods of cure and if stress relaxation is so large as shown in the present experiments this may preclude the use of C. N S L. resins when continuous loading is anticipated.

The modulus of elasticity of the C. N. S. L. resin was found to be rather low. The work was done on effect of repeated loading/unloading cycles of stress (perhaps the first to be done on adhesive gels) and this confirmed the preference of paraformaldehyde to formaldehyde for this adhesive. They also suggested that possibly an oscillatory schedule in the manufacture of articles using this resin will be useful.

INVESTIGATION OF PHYSICAL AND CHEMICAL FACTORS INFLUENCING THE ENZYMIC PROPERTIES OF PAPAIN*

B D Atreya
Tara Kuter Arya Puri Muzaffarnagar

Papain is a proteolytic enzyme of great commercial and academic interest. It shows a number of enzymic properties Its activity has been found to depend upon a variety of factors physical as well as chem cal Investigation of some such factors is the object of the present thesis

The thesis comprises an introduction and four chapters. The first chapter deals with the study of the proteolysis of different protein substrates by papain with special reference to the influence of the concentration of substrate and nature of the buffer under different pH conditions, on this proteolysis. In the first section, the effect of seven different buffers viz. (i) boric acid-succinic acid-sodium sulphate-borax, (ii) disodium hydrogen phosphate-citric acid, (iii) succinic acid borax, (iv) acetic acid-sodium ace tate, (v) boric acid-borax, (vi) potassium dihydrogen phosphate-borax and (vii) potassium dihydrogen phosphate-disod um hydrogen phosphate, on the hydrolytic property of papain against casein as substrate has been studied at different pH'S and for different periods. It has been found that neutral or faintly alkaline solutions favour the hydrolysis of casein by papain. Most of the hydrolysis takes place during the first 48 hours, after which the amino acid content often remains the same.

The soluble proteins of the seeds of *Phaseolus radiatus Phaseolus mungo Cajanus indicus* and *Lens esculentum* have been tried as substrates for papain and the results are described in the second section of Chapter I Regularly varying concentrations of the substrate have been taken and boric acid borax buffer at pH = 7 09 7 60 and 8·08 has been used It is observed that, the soluble proteins of *Phaseolus radiatus Cajanus indicus* and *Lens esculentum* are broken down by papain within a range of pH = 7·09 to pH = 8·08, but the proteolysis of the soluble proteins of *Phaseolus mungo* is optimum only at pH = 7·60 and it is minimum at othe pH Moreover within the range of pH = 7·09 to pH = 8·08, the mixture cotaining low concentrations of the above proteins show greater proteolysis at lower pH values of the mixture, and the mixture containing h gh concentrations of the above proteins show greater proteolysis at higher pH values of the reaction mixture. At a const ant pH greater proteolysis is observed with an increase in the concentration of the substrate and this is true for almost all the mixtures within the concentration limits examined.

*Summary of the thesis submitted for the Degree of Doctor of Philosophy of Agra University Agra.

A study of the influence of casein concentration on the papain-casein reaction at different hydrogen-ion concentrations, employing boric acid-borax and citric acid phosphate buffers, has been made in the next section of the chapter. Five different concentrations of casein viz., 1% 2%, 3%, 4% and 5% have been tried as substrate. It is observed that the hydrolysis of casein proceeds satisfactorily at pH=7·09 with 1 2, 3 and 5% concentrations of casein when boric acid-borax is used as buffer. With increasing pH value the mixtures containing smaller concentrations of casein become almost static with very little change in their hydrolysates concentration while media containing high concentrations of casein show strong decrease in the initial concentrations of the hydrolysates during the first 24 hours, which is followed by hydrolysis of the protein during the next 24 hours at pH=9·24 after which the hydrolysates concentration of all the reaction media falls rapidly. In reaction mixtures containing citric acid-phosphate buffers the initial hydrolysates concentration is almost zero and it increases during the first 72 hours at pH=7 and 8, and becomes almost steady afterwards. These studies of proteolysis with different concentrations of casein indicate that different enzyme-substrate complexes are formed at different conditions of casein concentration and that, these complexes are differently affected by the pH and also by the buffer employed in the experiment.

To study the influence of certain activators and inhibitors on the proteolytic activity of papain is the theme of the second chapter which includes three sections. The first section deals with the effect of hydrogen peroxide, potassium persulphate, ascorbic acid and glucose on the activity of papain when it has been activated by potassium cyanide hydrogen sulphide, sodium thiosulphate or urea. It is observed that sodium thiosulphate works as a weaker activating agent than potassium cyanide, hydrogen sulphide or urea. The presence of hydrogen peroxide or potassium persulphate inhibits the activation of papain by all the four reagents tried. Glucos and ascorbic acid promote the activity of papain in presence of hydrogen sulphide or urea but not in the presence of potassium cyanide or sodium thiosulphate.

The effect of copper sulphate, silver nitrate, gold chloride mercuric chloride and lead nitrate, on the activity of papain against the soluble proteins of *Phaseolus radiatus* seeds as substrate and the influence of 8-hydroxyquinoline on this proteolysis has been studied in the second section of this chapter. It is observed that, these metallic ions inhibit the proteolytic activity of papain, but to different degrees. On comparing the inhibiting effect of copper sulphate silver nitrate and gold chloride it is found that silver nitrate is the strongest and gold chloride the weakest inhibitor of the three. The inhibition by mercuric chloride is greater than that by lead nitrate. Addition of increasing volumes of 8-hydroxyquinoline solution counteracts the inhibiting influence of the above ions.

In the next section of this chapter the effect of adding ripe papaya extract on the proteolytic property of papain has been studied. The concentration of papain in papaya fruits is maximum just before ripening and during ripening its quantity continuously falls till it is zero when the fruit is fully ripe. It is not established whether the quantity of papain actually diminishes in ripe papaya leading to reduced proteolytic activity or this decrease is due to deactivation of the enzyme by some inhibitors formed during ripening. It is here observed, that the juice of the ripe papaya fruit acts as an inhibitor of proteolytic activity of the enzyme present in the extract of raw papayas. The soluble proteins of the powdered seeds of *Cicer arietinum* were used here as substrate for the enzyme under study. The inhibitory effect of the juice from ripe papaya fruits is found to be more pronounced when smaller volumes of it are tried as inhibitors.

The influence of some organic activators and inhibitors on the proteolytic activity of papain has been studied in the third chapter which comprises three sections. The first section deals with the influence of amines on the proteolysis of casein by papain. The amines tried are aniline, aniline hydrochloride, aniline sulphate, o-toluidine, m-toluidine, p-toluidine, α-naphthylamine, β-naphthylamine, phenylhydrazine, phenylhydrazine hydrochloride, dimethylaniline, diethylaniline, diphenylamine, piperidine pyridine triethylamine and o-phenylenediamine. It is observed that, these monoamines can be classified into three groups according to their influence on the proteolytic activity of papain against casein. The first group consists of such amines which decrease the hydrolysis of protein in the beginning, but increase it afterwards. These are aniline aniline hydrochloride, o-toluidine, and phenylhydrazine. The second group includes such amines which indicate activation in the early period of hydrolysis, but have definite inhibitory influence afterwards. This group includes m-toluidine α-naphthylamine, phenylhydrazine hydrochloride, dimethylaniline, and o-phenylenediamine. The third group of amines has no influence on the hydrolysis of casein by papain, and includes aniline sulphate, p-toluidine, β-naphthylamine, diethylaniline, diphenylamine, piperidine, pyridine and triethylamine.

This aspect of amine influence on the proteolysis by papain is extremely interesting because of its apparent resemblance to the biological property of counteracting the effect of a change in the environment. The counteracting inhibitory influence produced in this mixture of papain for amine, which first produces a null point and then converts it to activation and, also the activating influence, formed in the reaction mixture containing papain for amine which first reduces the activation to zero and then makes it inhibitory closely resembles the typical biological property. The detailed investigation of the mechanism of this counteracting of an external chemical factor shall be of great interest in the study of the Origin of Life to explain the development of the property of adaptability in the protoplasm.

Study of the influence of amino acids and amines on the proteolytic activity of papain has been aimed at in the second section of this chapter. Many proteins are known to react with peptides and amino acids forming definite complexes. The protein molecules thus modified, undergo changed chemical proteolysis by enzymes. It was naturally of interest to study the proteolysis of casein by papain in the presence of some amino acids. Glycine and L-tyrosine have been tried here. It is observed that their presence in the mixture containing casein and papain enhances the proteolysis of casein at pH $= 7{\cdot}09$. But, at pH $= 8{\cdot}08$ though glycine indicates an activation throughout the period of 120 hours the mixture containing L-tyrosine shows a definite inhibition in the beginning and towards the end.

In some experiments described under this section, amino acids either singly or in combination with other amino acids, have been used as the substrate for papain in the presence of β-naphthylamine, phenylhydrazine hydrochloride and dimethylaniline. The amino acids tried as substrates are (i) glycine and (ii) glycine glutamic acid mixture. It has been found that, if glycine is the substrate, and the reaction mixture contains papain, and β-naphthylamine, phenylhydrazine hydrochloride or dimethylaniline, there is a considerable decrease in the amino acid content of the mixture at both the pH values 7·09 and 8·08 tried in the experiment. This decrease is more pronounced at pH $= 8{\cdot}08$. The diminution in acidity is due to the formation of peptides which subsequently get hydrolysed after 48 hours. No such decrease in the amino acid content is observed if the mixture contains glycine together with glutamic acid at both the pH mentioned above.

The effect of anthraquinone, benzoquinone, hydroquinone and resorcinol, added individually on the hydrolysis of casein by papain has been the subject for study in the next section of this chapter. It has been observed, that quinones and phenolic compounds act as inhibitors in the hydrolysis of casein by papain. The enzyme develops some resistance towards the end against the inhibiting effect. Larger concentrations of casein do not favour the action of the inhibitor. *Hydroquinone and benzoquinone* work better as inhibitors than anthraquinone or resorcinol. Keeping the quantity of hydroquinone constant low concentrations of the enzyme do not indicate appreciable inhibition but, if the concentration of casein is increased, the inhibition is stopped. This shows that hydroquinone reacts with casein forming casein-hydroquinone complex which is not easily hydrolysed by papain. *However* with the increase of the period of enzymic action, the metabolites formed by the hydrolysis of free casein take up the hydroquinone group of the casein-hydroquinone complex and leave the casein free. This is indicated by the fact that the inhibition caused by hydroquinone diminishes with increasing period of enzymic action.

The last chapter of the thesis includes a study of the influence of sequence of addition of some substances on the action of papain, and ...

tion of the formation of papain-enzymoid. In the first section dealing with the effect of sequence of addition, an aqueous extract of gram flour (powdered seeds of *Cicer arietinum*) or a casein solution has been tried as the substrate for papain, and aniline, aniline hydrochloride, aniline sulphate or triethylamine as inhibitors. It is observed that there is marked influence of the sequence in which enzyme and substrate are added to the inhibitor. Generally there is greater inhibition when enzyme is added in the last to the substrate—inhibitor complex.

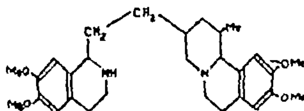
Gurwitsch reported in 1938 about the formation of 'enzymoids' when enzymes are allowed to stand in very dilute solution of glycine for varying periods. This possibility was investigated for papain in the second section of this chapter. The milk-clotting property of papain was tried as a basis for establishing the formation of papain-enzymoid'. It was observed that if a dilute solution of papain is further diluted with 0·5% glycine—H_2S solution the milk-clotting property of the enzymic solution decreases at first with the increase in dilution, but after a few dilutions, the milk-clotting property increases with increasing dilution. This enhancement in the milk-clotting property is greater when the solution is allowed to stand in diffused light for 30 or 60 minutes after dilution than the case in which the freshly diluted solution is added to milk without keeping. It has been postulated that the increase in milk-clotting property of the enzyme at such great dilutions as tried here, is due to the formation of some milk-clotting compound which has been named 'enzymoid of papain.

SEARCH FOR NEW AMOEBICIDES*

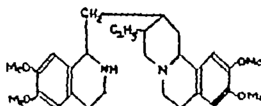
S N SAWHNEY†
Madhava College Ujjain

Amoebiasis—a disease caused by protozoa *Entamoeba histolytica* has been a major health hazard in the tropics The drugs that are available are emetine, emetine bismuth iodide, chiniofon vioform diiodoquin, carbarsone thiol derivatives of carbarsone oxide, milibis and chloroquin. Emetine stands out as the most potent of all the drugs tested clinically and thus remains outstanding in the treatment of amoebic dysentery hepatic abscess and chronic amoebiasis. Nevertheless, it cannot be classed as an ideal drug because of its cumulative toxic action its narrow margin of safety and its inability to kill the encysted form of *E. histolytica*. This necessitates the search for a substitute far superior to emetine.

Emetine was formulated by Brindley and Pyman (*J Chem. Soc.* 1927 1067) as (I) but is now more correctly represented by (II) as shown by the investigations of Robinson (*Nature*, 1948 **162** 524) Paller *et al* (*Monatsh* 1949 **80** 94) and Battersby and Openshaw (*Experientia* 1949 **5** 398 *J Chem. Soc.* 1949 3207) Emetine is more active *in vivo* than *in vitro* which furnishes every reason to believe that emetine does undergo some degradational changes in the body though attempts to locate this change have been futile.



(I)



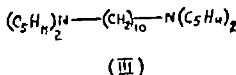
(II)

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* Summary of the thesis submitted for the Degree of Doctor of Philosophy of Agra University Agra.

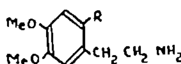
† Present address Lecturer in Chemistry Kurukshetra University Kurukshetra.

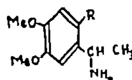
Based on the hypothetical fission of the emetine molecule, along different lines of cleavage, several amines have been synthesised and examined for their amoebicidal activity (Child and Pyman *J Chem. Soc* 1929 2010 1931 36 Pyman *Chem. & Ind.* 1937 56 789 Goodson *et. al Brit. J Pharmacol.*, 1948, 3 49 Goodwin *et al ibid*, 1948 3 63 Sugasawa, *J Pharm Soc Japan*, 1949 69 8 Hall *et al J Chem. Soc.* 1950 1842 1952 149 Mahboob and Dhar *J Sci Industr Res* 1955 14B 1 Osbond, *J Chem. Soc* 1951 3464 1952, 4785 1959 2157 Paul and Nitya Anand, *J Sci. Industr Res* 1958, 17B, 219 Fancher *et al J Amer Chem. Soc* 1958 80 1451 Sen and Arora, *Jour. Indian Chem. Soc* 1959 36 349) Only a few compounds especially ∝—ω—tetra—n—amyldiaminodecane (T A D D III) prepared by Pyman *loc. cit.*,) showed a high order of activity *in vitro* but only feeble activity *in vivo* (Goodwin *et al Brit. J Pharmacol.* 1948 3 44)



(III)

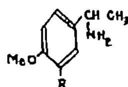
The same line of approach was adopted by Kachru and Pathak (*Jour Indian Chem. Soc* 1957 34, 611 768) who synthesised β and ∝-(2-alkyl-4.5—dimethoxyphenyl)— ethylamines (IV) and (V) as possible hypothetical cleaved fragments of the emetine formula. These amines were tested by Kaushiva (*J Sci Industr Res* 1957 16C, 224) who reported the highest *in vitro* activity in hexyl derivatives (comparable to emetine) Moreover the β—amines



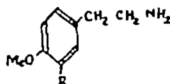


(IV) (V)

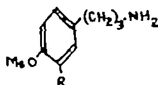
were found to be more active than their ∝-analogues. Based on these observations it was considered worthwhile to prepare structural analogues of the above amines with different phenolic moiety and to examine their amoebicidal activity This would help in locating the seat of activity in the amines described by Kachru and Pathak (*loc cit.*) Moreover since the β-substituted amines were found to be comparatively more active than their ∝-isomers, the activity would be expected to increase by removing the basic centre still further away from the nucleus by one and two carbon atoms. The following four types of amines have been synthesised for this purpose.



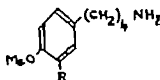
(VI)



(VII)



(VIII)

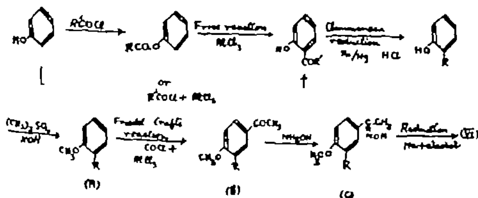


(IX)

The thesis contains the synthesis of (VI) (VII) (VIII) and (IX) described in Parts I II III and IV respectively

Synthesis of α (3-alkyl-4-methoxyphenyl)-ethylamines (VI) R = CH_3, C_2H_5, n-C_3H_7 n-C_4H_9 n-C_5H_{11} n-C_6H_{13} and n-C_8H_{17}

These amines were synthesized by the route as shown below

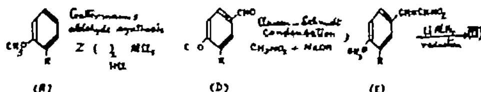


o-Cresol was methylated with dimethyl sulphate in the usual manner to obtain o-cresyl methyl ether (A, R = CH_3) To obtain other o-alkyl anisoles viz., (A, R = C_2H_5, n-C_3H_7) from phenol, the latter was esterified with appropriate acid chloride and subjected to Fries reaction. The resulting o-hydroxy ketones were recovered by steam-distillation, reduced according to the Clemmensen procedure and then methylated The higher alkyl anisoles were obtained by Friedel-Crafts acylation of phenol, followed by Clemmensen reduction and methylation.

o-Alkyl anisoles (A) were subjected to Friedel-Crafts condensation with acetyl chloride to yield the ketones of the type (B). These were converted into the oximes and reduced by sodium and alcohol to the corresponding amines (VI). The amines were examined as their hydrochlorides.

Synthesis of β-(3-alkyl-4-methoxyphenyl)-ethylamines (VII) R=CH_3, C_2H_5, n-C_3H_7, n-C_4H_9, n-C_5H_{11}, n-C_6H_{13} and n-C_8H_{17}

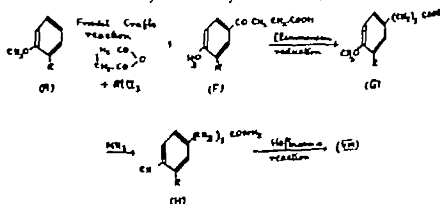
The following route has been adopted for the synthesis of these amines



o-Alkyl anisoles were subjected to Gattermann's aldehyde synthesis as modified by Adams and Levin to obtain 3-alkyl-4-methoxybenzaldehydes. This procedure involves the use of zinc cyanide instead of the hazardous hydrogen cyanide. The resulting aldehydes (D) were condensed with nitromethane in the presence of dilute alkali to yield nitrostyrenes (E). Some nitrostyrenes separated as yellow crystalline solids while others were isolated as viscous oils which were as such reduced with lithium aluminium hydride. The amines (VII) were isolated either in the form of their hydrochlorides or as picrates.

Synthesis of γ—(3-alkyl-4-methoxyphenyl)-n-propylamines (VIII) R=CH_3, C_2H_5, n-C_3H_7

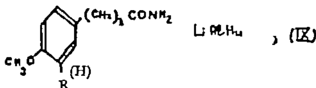
These amines were synthesised by the following route



o-Alkylanisoles were condensed with succinic anhydride in nitrobenzene solution in the presence of anhydrous aluminium chloride to yield the corresponding ketoacids (F). Solvents other than nitrobenzene e. g. carbon disulphide and s-tetrachloroethane were also tried but the yields were very poor. The keto acids were reduced by the Clemmensen reduction in the usual m

in toluene solution. Subsequent conversion to the amide was achieved by passing a current of dry ammonia through the molten acid at 200° for two hours. The production of the amines (VIII) from the amides was done by the application of Hofmann's hypochlorite reaction.

Synthesis of δ-(3-alkyl-4-methoxyphenyl)-n-butylamines (IX) R = CH_3, C_2H_5, n-C_3H_7 and n-C_4H_9.



γ-(3-alkyl-4-methoxyphenyl) n-butyramides (H R = CH_3, C_2H_5 n-C_3H_7 and n-C_4H_9) have been obtained as intermediates in the synthesis of γ-(3-alkyl-4-methoxyphenyl)-n-propylamines These amides were reduced by lithium aluminium hydride in anhydrous ether to the corresponding amines (IX) The amines were isolated as hydrochlorides.

Pharmacological findings

Amoebicidal testing (*in vitro*) of some of the selected compounds of all the four series was undertaken at the Central Drug Research Institute, Lucknow δ-(3-Alkyl-4-methoxyphenyl)-n-butylamines have been found to be promising The activity increases in general with the rise in the molecular weight in all the series. Amoebicidal testing (*in vivo*) of these amines is under investigation.

THE HEAD-SKELETON OF *SILONIA SILONDIA* (HAM.)

MANJULA RASTOGI

Zoology Department Meerut College, Meerut

INTRODUCTION

On the advice of Dr B M Sinha investigations on the head skeleton of Indian Schilbeid Fishes have been undertaken. The family is interesting, as it is known from Oriental and Ethiopian regions only It shows marked affinities with the family Bagridae on one hand and with family Silondae on the other

The present work is devoted to *Silonia silondia* and investigations on other Indian forms will be taken in due course.

It is my pleasant duty to thank Dr B. M. Sinha for his guidance in the work and to the authorities of the college for providing the necessary facilities.

THE HEAD SKELETON

The skull of *Silonia silondia* is a complex structure composed of the replacing and investing bones. It is platybasic and fenestrated.

The skull is wedge-shaped with three median fontanelle and a pair of temporal fossae. The fontanelle lie one behind the other in the ethmoidal, frontal and occipital regions, while the temporal fossae are restricted to the occipital and auditory regions.

The Occipital Region

The occipital region consists of the usual four replacing bones, the supraoccipital above basioccipital below and exoccipitals on the sides.

The *Supraoccipital* (I II III IV and V 1) is the largest of the occipital bones and projects behind into the supraoccipital spine. The bone may be distinguished into a vertically expanded part and the horizontal part. The vertically expanded part is cleft in front and is produced into a pair of processes, which articulate with the frontals. The two processes and the cleft in between form the posterior two-third of the occipital fontanelle. The horizontal part contributes to the formation of temporal fossa articulating with the adjacent frontal, sphenotic, pterotic and exoccipital From the junction of the vertically expanded and horizontal parts and from about the middle of the bone is given, on either side, a backwardly directed process, which unites with a process from the epiotic. The two processes together form a bridge over

the temporal fossa and enclose the posterior vertical canal of internal ear. Behind the origin of the process, the vertically expanded part is perforated by a cleft passing from one side to the other which is for the passage of the occipital commissure of lateral line system. Beneath the bone lodges a part of the anterior vertical canal of internal ear

The *exoccipital* (I II III IV and V 2) is an irregular bone, which also takes part in the formation of foramen magnum It gives off a vertical neural plate, an outwardly directed inclined plate and a feeble inwardly running horizontal plate. The *neural plates* from the two exoccipitals meet dorsally to form the dorsal and lateral boundaries of the *foramen magnum*. The *inclined plate* is directed downwards and encloses a vacuity between it and the main bone, through which pass the glossopharyngeal and vagus nerves. The inclined plate meets the neural plate on the outer side and the two enclose a foramen for the posterior vertical and horizontal canals of internal ear From the inner side of neural plate arises the *horizontal plate* which meeting the one of the other side forms the floor of foramen magnum and the roof of cavum *sinus imparis*. On the posterior side of the bone and along the inner aspect of the vacuity is an aperture for the first spinal nerve and below this aperture is an articular surface for the basioccipital.

The bone articulates with the supraoccipital prootic, pterotic, epiotic and the basioccipital.

The *basioccipital* (III IV and V 3) is well formed and bears a well developed occipital condyle at its posterior end. Beneath the condyle is given backwards a pair of accessory articular processes. On the dorsal side of bone are three depressions, a well developed median and two lateral. These depressions together with similar depressions beneath the exoccipitals enclose the sinus endolymphaticus and pars inferiores of internal ear The ventral surface of the bone is somewhat corrugated. Along this aspect on either side of the bone is a depression for the inferior limb of posttemporal.

The bone articulates with the prootics and exoccipitals and it laterally sutures with the parasphenoid.

The Auditory Region

The auditory region is formed of four replacing bones the prootic, epiotic, sphenotic and pterotic, the opisthotic being absent.

The *prootic* (III IV and V 5) is a flattened bone on the ventral side of auditory region. Its inner end in the posterior half is greatly thickened and is produced upward into a projection from the inner side of which is given a transversely directed ridge. Along the posterior side of this ridge lies a part of the horizontal canal of internal ear and through a tunnel below the ridge passes the anterior vertical canal of the ear Behind the ridge and on the

inner side of the projection of the bone is a depression for the recessus utriculi. Along the anterior face the bone is notched and the notch forms a part of the trigeminofacial fenestra. Behind the notch is a foramen through which emerges hyomandibular trunk of facial nerve. The two prootics articulate with each other in the middle line by their projections only A small niche is left between the projections and the parasphenoid which represents the vestigial posterior myodome.

The prootic articulates with the basioccipital exoccipital pterotic, sphenotic and pleurosphenoid.

The *epiotic* (I II III IV and V-6) lies on the postero-lateral side of the auditory region. It is formed of a ventral plate constituting partially the floor of temporal fossa and an upwardly directed process The bone is traversed by a tunnel for the posterior vertical canal of internal ear The epiotic articulates by its body with the pterotic supratemporal posttemporal and exoccipital, and by its process with the supraoccipital

The *pterotic* (I II III IV and V 4) is a flattened squarish bone on the postero-lateral side of auditory region From the lateral side of the bone is given inwards a crest, which articulates with the sphenotic and supratemporal The bone is traversed by a tunnel through which passes the horizontal canal of internal ear On the ventral aspect of the bone is a shallow groove which is a continuation of a more prominent groove on the sphenotic. The bone articulates with the epiotic, exoccipital, prootic and sphenotic.

The *sphenotic* (I II III and V 7) is the largest bone of the auditory region, situated in the antero-lateral part of the region. It consists of a thick basal part and a forwardly directed apical plate. The basal part is slightly depressed and forms a part of the temporal fossa. Along its ventral side is a groove which together with that of pterotic gives articulation to the head of hyomandibula. The apical part contributes to the formation of the boundary of orbit and a notch on its outer side gives articulation to the last suborbital bone. The basal part articulates with the frontal, supra-occipital and pterotic, while the apical part articulates with the frontal and lateral ethmoid

The *supratemporal* (I II and V 13) is a small flat bone, which is firmly articulated to the cranium and roofs partially the post temporal fossa It articulates with the pterotic, epiotic and posttemporal.

The *posttemporal* (I II III IV and V 12) is a well developed bone, which secondarily fuses with the cranium and becomes more the part of the skull than the girdle It consists of the main part and two limbs, a superior and an inferior The main part of the bone carries a deep notch in which is received the dorsal limb of the cleithrum. The superior limb is stout and articulates with the supratemporal epiotic and the pterotic, while the inferior limb is weak and abuts on the articular facet presented by the basioccipital

The Sphenoidal Region

The Sphenoidal region is ossified by the paired pleurosphenoids and frontals and the unpaired orbitosphenoid and parasphenoid. The basisphenoid is absent. The pleurosphenoids and orbitosphenoid are replacing bones, while others are investing ones

The *frontal* (I II III IV and V-8) is a flat fenestrated bone on the dorsal side of sphenoidal region. Its inner end drops down into a crest, which abuts on the pleurosphenoid and orbitosphenoid. The posterior end of the bone is depressed to form part of the temporal fossa. Mesially with the bone of other side it encloses a small fontanelle. The bone articulates with the ethmoid, lateral ethmoid sphenotic and supraoccipital.

The *pleurosphenoid* (V 10) is a wing like bone in the postero-lateral part of sphenoidal region Its upper surface is deeply notched contributing to the formation of optic foramen On its lower side and at the posterior end is a notch which forms part of the fenestra for trigeminofacial complex. The bone articulates with the orbitosphenoid, frontal, sphenotic and prootic.

The *orbitosphenoid* (III IV and V 11) is a compound bone formed by the fusion of the paired elements It is situated in the anterior part of the sphenoidal region The bone is broad in front and narrow behind and it is hollow like a drain pipe for the passage of olfactory tracts. On the ventral side of it is a longitudinal groove for the stem of parasphenoid. The bone articulates with the lateral ethmoids pleurosphenoids, frontals, and parasphenoid.

The *parasphenoid* (III IV and V 9) is a long bone on the floor of the cranium extending from the ethmoidal to the occipital region. It consists of a rhomboidal body and a long forwardly directed stem. The body is elongated in the antero-posterior direction and on its lateral side is a small notch which takes part in the formation of optic foramen The body articulates with the basioccipital prootics sphenotics while the stem with the orbitosphenoid and vomer

The Orbital Region

The orbits are large and each is bounded by the lateral ethmoid, sphenotic and four suborbitals The suborbitals are splint like bones which extend in a chain in front below and behind the eye. Through the suborbitals passes the infraorbital trunk of lateral line system.

Of the suborbitals the fourth is the largest, the third and second are small and the first is usually developed

The Ethmoidal Region

The ethmoidal region includes the unpaired ethmoid and vomer and the paired lateral ethmoids, nasals and lacrymals. The lateral ethmoids are the replacing bones while the remaining are investing ones

The *ethmoid* (I II III IV and VI 1) lies on the dorsal side of the ethmoidal region. It is a flattened plate like bone produced anteriorly into a pair of dorsal horns The bone separates behind into a dorsal and a ventral part. The dorsal part is very well developed and it is separated by a median ethmoidal fenestra into two processes, which articulate with the frontals. The ventral part is small and is produced in front into a pair of feebly developed ventral horns

The dorsal and ventral parts are separated by a recess which is divided by a median ridge into two lateral cavities forming the anterior part of nasal capsules. The bone articulates with the premaxillae, vomer and nasals. Its dorsal part articulates with the lateral ethmoids and frontals and the ventral part with the lateral ethmoids and vomer

The *lateral ethmoid* (I, II III IV and V-2) lies on its side of the ethmoid and frontal It is a large fenestrated bone which consists of an anterior head and a backwardly directed broad plate The anterior extremity of the head is depressed to form the posterior part of olfactory capsule On the inner side of the head is a vacuity which forms part of the olfactory capsule From the outer side and about the middle of the bone arises the antorbital process, which forms the anterior boundary of the orbit.

The bone articulates with the lacrymal palatine, ethmoid frontal, vomer orbitosphenoid, ectopterygoid and sphenotic.

The *vomer* lies on the ventral side of the ethmoidal region. It is a T-shaped bone consisting of a horizontal and backwardly directed limb The horizontal limb bears vomerine teeth below The backwardly directed limb is slender and tapering and lies in the groove below the parasphenoid. The bone articulates with ethmoid ectopterygoids lateral ethmoids and parasphenoid.

The *nasals* are splint-like tubular bones superficially applied on the inner side of olfactory capsule. Each lies along the outer margin of the ethmoid and in it terminates the supraorbital canal of lateral line system.

The *lacrymal* is a small irregular bone which lies above the anterior head of the palatine and base of maxilla. It is applied to the front end of first suborbital. In the bone terminates the infra orbital canal of lateral line system.

The Visceral Skeleton

The visceral skeleton consists of seven arches, the mandibular hyoid and five branchial arches, which encircle the buccal cavity and pharynx.

The Mandibular Arch

The mandibular arch is well developed and each half of it is divided into the palatopterygoquadrate bar and Meckel's cartilage. The palatopterygoquadrate bars form the upper jaw while the Meckel's cartilages give rise to the lower jaw The palatopterygoquadrate bar is formed of five replacing bones, the palatine, ectopterygoid entopterygoid, metapterygoid and quadrate and two investing bones the premaxilla and maxilla. The Meckel's cartilage is ossified by two bones the angular and dentary which are partly replacing and partly dermal.

The *palatine* (I II III and IV 11) is a small bone which lies on the inner side of the maxilla It joins by front end with the maxilla and lateral ethmoid while its hind end is free

The pterygoid is absent and in its place are the entopterygoid, ectopterygoid and metapterygoid.

The *entopterygoid* (VI 15) is a small comma-shaped bone, which is attached to the front end of the metapterygoid

The *ectopterygoid* (I III IV and VI 13) is a curved bone with the broad anterior and tapering posterior end The bone bears teeth on the lower side, which merge into those of vomer It articulates with the vomer metapterygoid and lateral ethmoid

The *metapterygoid* (I IV and VI-8) is a large plate-like bone notched in front It articulates in front with the lateral ethmoid and behind articulates with the hyomadibula and quadrate by interdigitation.

The *quadrate* (I IV and VII 3) is more or less rectangular bone situated below and on outside of the hyomandibula Its lower apex is thick and bears an articular condyle for the lower jaw The bone articulates with the metapterygoid hyomandibula and preoperculum

The *premaxilla* (I II III IV and VI 14) is a stout flattened bone, which together with the premaxilla of the other side forms the upper gape of mouth. On the upper side of the bone is a shallow concave articular surface for the ethmoid and on the lower surface run a single row of teeth. The bone gives articulation to the lacrymal and maxilla from its outer side.

The *maxilla* (I II III and VI 10) is a small edentulous bone which takes no part in the formation of the jaw It has a bifid head and a backwardly directed process. It is attached to the premaxilla and palatine by the two

knobs of head The bone is vestigial as the maxillary barbel which it supports in catfishes, is missing in the fish

The *angular* (I IV and VII 2) is a stout proximal bone of the lower jaw It bears in its proximal part a facet for the quadrate and articulates distally with the dentary

The *dentary* (I IV and VII 1) is an equally stout, but curved bone developed around the anterior two-third of Meckel's cartilage. It meets in front in a symphysis with that of the other side and behind it ends in a fork which encloses the distal end of the angular The upper surface of the bone is provided with mandibular teeth running in three rows

The Hyoid Arch

The two halves of hyoid arch meet in the midventral line. Each half consists of the hyomandibula and the hyoid cornu.

The *hyomandibula* (I IV and VII 5) is a prominent flattened bone, which hangs the jaws from the cranium. On the upper side it glides by a long head in the groove on the sphenotic and pterotic. On its posterior side is a knob for articulation of the operculum and above the knob is a small ridge, which gives articulation to the preoperculum The upper surface of the bone is provided with a foramina for the hyomandibular trunk of facial nerve. The hyomandibula articulates with the metapterygoid quadrate operculum and preoperculum. The articulation of the jaws with the cranium is through the hyomandibula and the suspension is *methyostylic*

The *hyoid cornu* hangs from the hind end of the hyomandibula by interhyal and consists of four bones the epihyal ceratohyal, and two hypohyals. The interhyal is a small rod like bone

The *epihyal* (VIII) is a flattened bone which articulates above with the interhyal and behind with the ceratohyal Along its posterior edge it carries two branchiostegal rays. The *ceratohyal* (VIII) is twice as long as the epihyal. It is attached by its broad hind end with the epihyal and by its narrow front end to the anterior hypohyal It supports along its posterior edge nine branchiostegal rays The *hypohyals* (VIII) are two small bones one behind the other each attached by the base with the ceratohyal and by its apex with the hypohyal of the other side The *branchiostegal rays* (VIII) are flattened curved rods, which are directed backwards and outwards. They increase in size and thickness from the first of the series to the last and support the branchiostegal membrane.

On the underside of the hypohyals directed backwards is the prominent *urohyal* It is connected in front to the anterior hypohyal by its stem and widening behind ends into three unequal processes, a median long and a small on either side. It bears a long ridge on the dorsal surface along either side of which articulates the cleithral bone.

Connected to the hyoid arch is a gill cover on either side. Each gill cover is formed of three bones the operculum, interoperculum and preoperculum, the suboperculum being absent.

The *operculum* (I IV and VII-6) is a flat triangular bone, the dorsal narrow end of which bears a socket for the condylar head of hyomandibula. Its upper surface is marked with radiating ridges commencing from its apex. The *interoperculum* (I IV and VII 7) is a scute-like bone applied to the posterior border of preoperculum Its posterior border is overlapped by the operculum. The *preoperculum* (I IV and VII-4) lies along the posterior edge of the hyomandibula and quadrate consisting of the main body and stem. The main body articulates by interdigitation with the posterior edge of quadrate and the lower posterior edge of hyomandibula The stem is applied to the upper posterior edge of hyomandibula.

The Branchial Arches

Of the five branchial arches the first four are of the usual type and the fifth is reduced The upper half of each arch is ossified by the pharyngobranchial and epibranchial and the lower half by the ceratobranchial and hypobranchial. The *pharyngobranchials* (VIII) are four small rod-like bones, which hang the branchial arches from the prootic The first and second pharyngobranchials are fused with their epibranchials while the fourth lies in front of the third. The *epibranchials* (VIII) are elongated bones which are grooved on the upper surface and bear gill rakers on the lower surface. Over the fourth pharyngobranchial and the third and fourth epibranchials is an oval pad bearing fine superior pharyngeal teeth. The *ceratobranchials* (VIII) are long curved bones more than twice as long as their epibranchials. Each is grooved on its lower side and provided with a double row of gill rakers on the upper surface. The *hypobranchials* (VIII) are ossified on the first and second branchial arches only They are in the form of oval pieces, which extend inwards from their ceratobranchials.

The *basibranchials* are ossified in the median cartilage which extend from the hypohyals to the fifth pair of ceratobranchials There are only two of them in the form of small rods, the first in between the first and second pair of hypobranchials and the second behind the second pair of hypobranchials.

The fifth arch is represented by its ceratobranchials only which are expanded and bear fine inferior pharyngeal teeth on their upper surfaces.

SUMMARY

1 The skull is platybasic highly fenestrated and with three median fontanelle and a pair of temporal fossae.

2. The opisthotic parietal, suboperculum, intercalar and sympl... are absent.

3 The supraoccipital does not participate in the formation of foramen magnum. It has a well developed occipital spine and two postero-lateral processes. The postero-lateral process articulates with the process of epiotic and the two together form a bridge over the temporal fossa enclosing the posterior semi-circular canal of internal ear

4 The prootics articulate through inwardly directed projections only They leave a small niche in between them and the parasphenoid and basioccipital, which represents the vestigial myodome The groove for the hyomandibula extends to both the sphenotic and pterotic.

5 The two orbitosphenoids fuse enclosing a canal for the olfactory tracts. The lateral ethmoid is a very much cancellate bone lodging the cavity for olfactory lobe. It articulates directly with the sphenotic, without the interruption of the frontal

6. The orbits are large and there are four suborbital bones The vomer is a T-shaped bone and is toothed.

7 The maxillo-palatine element is not well developed as the maxilla is vestigial, and maxillary barbel is degenerate

8 The metapterygoid is very well developed and ectopterygoid is toothed

9 The angular and dentaries are alone developed on lower jaw

10 The ramus of hyoid arch has two hypohyals an anterior and a posterior The basihyal is absent. The first and second pharyngobranchials are fused with their epibranchials. Over the fourth pharyngobranchial and the third and fourth epibranchials is an oval pad bearing the superior pharyngeal teeth. The fifth branchial arch is reduced to a pair of ceratobranchials, which bear inferior pharyngeal teeth.

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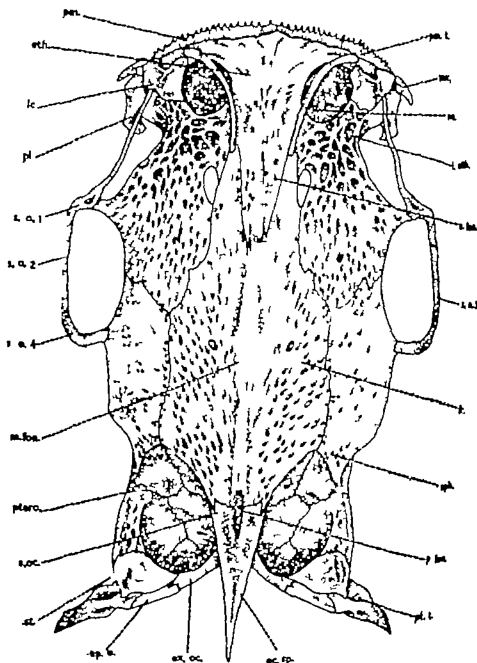
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Lateral View of the Skull

ang. angular; *a. fon.*, anterior fontanelle; *dent.*, dentary; *ep. o.*, opiotic; *ec. pter.* ectopterygoid; *eth.*, ethmoid; *ex. oc.* exoccipital; *f.* frontal; *h. m.* hyomandibula; *i. op.* interoperculum; *le.*, lacrymal; *l. eth.*, lateral ethmoid; *m. fon.*, median fontanelle; *m. pter.* metapterygoid; *mx.*, maxilla; *na.*, nasal; *op.* operculum; *p. fon.*, posterior fontanelle; *pl.* palatine; *pm.* premaxilla; *ptero.*, pterotic; *pt. t.* posttemporal; *pr. op.* preoperculum; *qu.* quadrate; *s.*, 1-4 first, second third and fourth suborbitals; *soc.* supraoccipital; *sph.*, sphenotic; *st.*, supratemporal.

Silonia silondia (Ham.)

Plate-II



Dorsal View of the Cranium

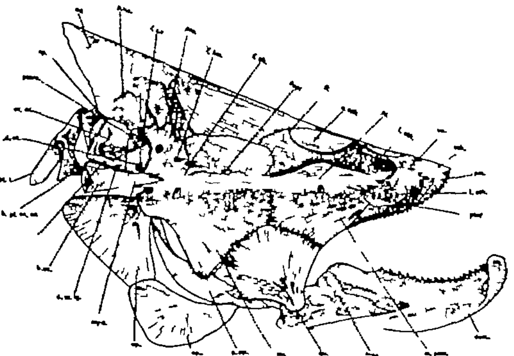
a. fon., anterior fontanelle *ep o.*, epiotic; *eth* thmoid; *exoc* exoccipital *fr* frontal *l. eth.* lateral thmoid *lc* lacrymal *m. fon.*, median fontanelle *mx* maxilla; *na* nasal *oc sp* occipital spine *P fon.*, posterior fontanelle *pm t.*, premaxillary teeth; *pl.*, palatine *pmx*, premaxilla *ptero.*, pterotic *pl. t.*, posttemporal *s. o.* 1-4 first, second, third and fourth suborbital; *s oc* supraoccipital *st.*, supratemporal *sph.*, sphenotic

Silonia silondia (Ham.) Plate-III

1 2 cm

Ventral view of the Cranium

a. a. p. accessory articular process; *b. oc.* basioccipital *ep. o.*, epiotic *ect. pter.*, ectopterygoid *eth.*, ethmoid; *ex. oc.*, exoccipital *fr.* frontal *f. hy. t.*, foramen for hyomandibular trunk; *f. tfc.*, foramen for trigeminofacial complex *gr. h. m.* groove for hyomandibular *l. eth.*, lateral ethmoid; *mx.*, maxilla; *oc. sp.* occipital spine; *or. sph.*, orbitosphenoid *pl.* palatine; *pm.*, premaxilla *pm. t.*, premaxillary teeth; *pro.*, prootic; *ps.*, parasphenoid; *ptero.*, pterotic; *pt. t.*, posttemporal; *s. 1-4* suborbitals first, second, third and fourth *sph.*, sphenotic; *vo.* vomer; *vo. t.* vomerine teeth.



Longitudinal Section of the Skull.

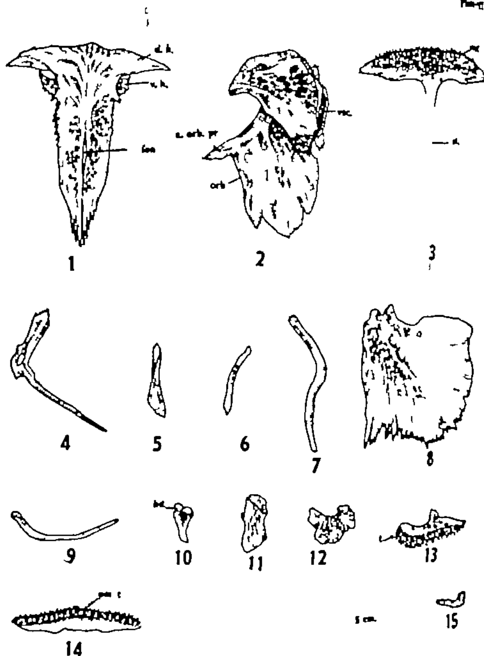
Silonia silondia (Ham.) Plate-V

Bones of the Skull Disarticulated

1 *supraoccipital*; 2. *exoccipital*; 3. *basioccipital*; 4 *pterotic* 5. *prootic* 6. *epiotic* 7 *sphenotic* 8. *frontal* 9 *parasphenoid* 10 *pleurosphenoid* 11 *orbitosphenoid* 12. *posttemporal* 13. *supratemporal*

a. a. p. accessory articular process *ar* arm; *b. p.* basal part; *bd.*, body; *cd.*, condyle; *ch.*, channel; *cr* crest; *c. s. i.*, cavum sinus imparis *d. ut* depression for utriculus *d. sc. lg* depression for sacculus lagina; *f. v.* foramina for vagus nerve *f. h. m.* foramina for hyomandibular trunk; *f. op.* foramina for optic nerve; *h. pl.*, horizontal plat *i. l.*, inferior limb; *i. pr* inner projection; *l. pr.*, lateral process; *nt* notch *orb* orbi *p. fon.*, posterior fontanelle *pr* process *sp.*, spine; *s. l.*, superior limb.

Silonia silondia (Ham.)



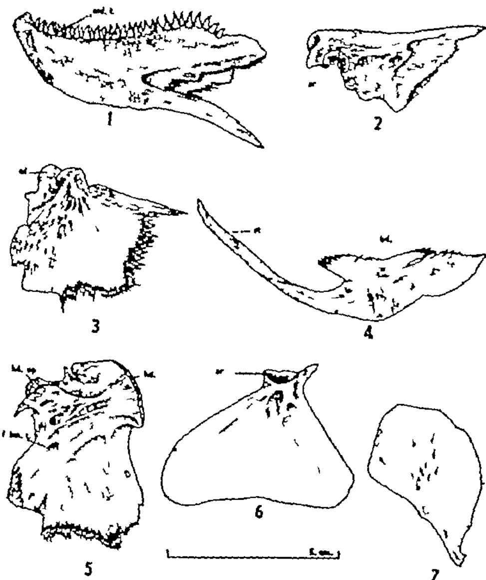
Bones of the skull Disarticulated

1 *ethmoid*, 2. *lateral ethmoid*, 3 *vomer* 4-7 *first, second, third and fourth suborbitals* 8. *supraorbital* 11 *pterygoid* 9 *nasal* 10 *maxilla* 11 *palatine* 12. *lacrymal*; 13. *supraorbital* 14 *premaxilla* 15. *ectopterygoid*

a. fon., anterior fontanelle; *a. orb. pr* antorbital process *d. h.*, dorsal horn and orbit stem; *v. h.*, ventral horn *vac* vaculty; *v. t.*, vomerine teeth.

<u>Silonia silondia</u> (Ham.)

Plate-VII



Bones of the Skull Disarticulated

1. *dentary* 2. *angular* 3. *quadrate* 4. *preoperculum* 5. *hyomandibula* 6. *operculum* 7. *interoperculum*.

ar. s., articular surface *bd.*, body; *cd.*, condyle *f. hm. t.*, foramen for hyomandibular trunk; *hd.*, head; *hd. op.*, head for operculum *md. t.*, mandibular teeth *st.*, stem

The Hyobranchial Skeleton

b. br., basibranchials; *b.r.* branchiostegal rays; *Cart.*, cartilage; *c. br.*, ceratobranchials; *c. hy.* ceratohyal; *e. br.* epibranchials; *e. hy.* epihyal; *g. r.*, gill rakers; *h. br.*, hypobranchials; *h. hy.*, hypohyals; *i. p. t.*, inferior pharyngeal teeth; *ph. br.* pharyngo branchials; *s. p. t.* superior pharyngeal teeth.

ENTOMOLOGICAL SURVEY OF THE HIMALAYA*

PART XXIV—FOURTH AND FINAL ANNOTATED CHECK-LIST OF THE INSECTS FROM THE NORTH WEST (PUNJAB) HIMALAYA.†

SANTOKH SINGH

School of Entomology St. John's College Agra.

This is the fourth check list of the series. This brings the total number of species to 2470 belonging to sixteen Orders and it is hoped that all the species so far known from the N W Himalaya have been listed

I take this opportunity for thanking Dr M S. Mani Deputy Director Zoological Survey of India, Calcutta, for help and keen interest in the work. Also I thank the authorities of St. John's College, Agra, for facilities for work.

Order COLLEMBOLA

FAMILY HYPOGASTRURIDAE

1 *Onychiurus kalitus* Baijal Agra Univ J Res (Sci.) 5
*Gramphu (Chandra Valley) 12000 ft.

2 *Xenylla sucta* Baijal Agra Univ J Res (Sci.) 5
*Rhala, 8835 ft.

3. *Friesea maxima* Baijal Agra Univ J Res. (Sci) 5
Gramphu (Chandra Valley) 12000 ft.

FAMILY MYDONIIDAE

4 *Sinella montana* Imms, Proc Zool Soc London, pl viii fig 48 pp. 101 102 (1912) Baijal, Agra Univ J Res. (Sci.) 5
Gramphu Kulti Nal, Chhatru Rhala 9000 ft. Marhi 12000 ft Hamta Gorge, 14000 ft. Badrinath Garhwal Himalaya Nilgiris.

FAMILY TOMOCERIDAE

5 *Tomocerus ocreatus* Denis Mus Heude Notes Ent. Chin. 12 (17) 220 (1948) Baijal Agra Univ J Res. (Sci.) (Suppl.) 4 760 (1955)
*Rhala, 10,000 ft. Marhi, 12000 ft. Manali-Rhala Road, 6000 ft. Rhotang Pass, 13500 ft. Gramphu 12000 ft. Chhatru 11500 ft. Peak to the West of Rhotang Pass 15000 ft. Purana Khokasr Nal 12500 ft. Indo-China.

* Contribution No. 91 from the School of Entomology St. John's College, Agra.

† The first three check-lists were published as parts IX, XII and XVII of the Entomological Survey of Himalaya, in *Agra Univ J Res* (Sci.) 4(2) 471 512 (1955) 4(Suppl.): 6 57 718 (1955); 5.

Order ODONATA

Family Libellulidae

1 *Nanophya katrainensis* Baijal Agra Univ J Res. (Sci.) (Suppl.) 4 :41 (1955)
*Katrain.

2. *Orthetrum pruinosum neglectum* (Rambur) Ins. Neurop p. 86 (1842) (*Libellula*) Baijal Agra Univ J Res (Sci) (Suppl.) 4 744 (1955).
*Kulu Mandi Road 3500 ft. Whole of India Ceylon Burma, Tibet, Indochina Hong Kong Nilgiris

3 *Orthetrum chrysostigma luzonicum* (Braur) Verh. Zool. bot. Ges. Wien, 18 169 (1868) Baijal Agra Univ J Res (Sci.) (Suppl.) 4 745 (1955).
*Manali 6500 ft. Throughout India, Ceylon, Philippines, South Asia, Java, and Sumatra.

4 *Orthetrum guptai* Baijal, Agra Univ J Res. (Sci.) (Suppl.) 4 746 (1955)
*Manali, 6500 ft.

Family Coenagriidae

5 *Coenagrion fallax* (Ris) Ent. Mitteil. 3(2) 47 (1944) Baijal Agra Univ J Res. (Sci.) (Suppl.) 4 747 (1955)
*Katrain Simal Hills Sikkim Assam Bengal Tibet.

6 *Archibasis sushmae* Baijal, Agra Univ J Res. (Sci.) (Suppl.) 4 : 747 (1955).
*Manali, 6500 ft.

Order EPHEMEROPTERA

Family Ephemeridae

1 *Ephemera consors* Eaton, J Asiatic Soc. Bengal, 60 (2) 412 (1892) Hafiz, RIM. 39 368 (1937)
*Kulu Sikkim

2 *Ephemera remensa* Eaton J Asiatic Soc. Bengal 60 (2) 410 (1892) : Hafiz, RIM. 39 368 (1937)
*Kulu. Mussourie.

3 *Ephemera sp* Eaton J Asiatic Soc. Bengal 60 (2) 413 (1892)
*Kulu.

Family Heptageniidae

4 *Epeorus psi* Eaton Trans. Linn. Soc. (2) 3 242 (1885) J Asiatic Soc. Bengal 60 (2) 413 (1892)
*Kulu.

5 *Epeorus sp* Eaton J Asiatic Soc. Bengal 60 (2) 413 (1892).
*Kulu.

Order PLECOPTERA

Family Perlidae

1 *Cryptoperla tarra* Needham, RIM. 3 190 (1909)
*Kulu.

2. *Neoperla indica* Needham RIM., 3 188 (1909)
*Kulu.

3 *Perla decemcelia* Pictet. Hist. Nat. Nevr Perlids pp 258-9 pl xxvii fig 1 2 (1847) Needham RIM 3 189 (1909)
*Kulu.

4 *Perla ione* Needham RIM. 3 187 (1909)
Kulu. Kurseong

Family Perlodidae

5 *Perlodes amabilis* Jewett, *Proc Nat Acad Sci., Allahabad 28 (B) 320-329*
*Manali 6000-6500 ft.

Family Nemouridae

6 *Capnia manu* Jewett, *Proc Nat Acad. Sci* Allahabad 28 (B) 320-329
*River Beas Valley near Marhi 11000 ft, Marhi 12000 ft.

7 *Leuctra* (*Rhopalopsole*) *magnicerca* Jewett, *Proc Nat Acad. Sci.*, Allahabad 28 (B) 320-329
*Kote 8000 ft.

8. *Nemoura* (*Amphinemoura*) *trisnatha* Jewett, *Proc Nat Acad Sci* Allahabad, 28 (B) 320 329
*Kote (Kulu Valley) 8000 ft.

9 *Nemoura* (*Nemoura*) *ampula* Jewett, *Proc Nat Acad. Sci.* Allahabad, 28 (B) : 320-329
*Rhala 8835 ft.

10 *Nemoura* (*Nemoura*) *cordata* Jewett *Proc Nat. Acad Sci* Allahabad 28 (B) : 320-329
Gramphu 11000 ft. Chandra Valley 11 12000 ft.

11 *Nemoura* (*Nemoura*) *punctata* Jewett *Proc Nat Acad Sci* Allahabad, 28 (B) 320-329
Gramphu 12000 ft. Beas Valley nea Marhi 11000 ft.

12 *Nemoura* (*Nemoura*) *punjabensis* Jewett, *Proc Nat Acad. Sci.*, Allahabad, 28 (B) 320-329
*Chhatru 11 12000 ft Rhala 9000 ft. 2 miles south of Rhala 10-11000 ft. Kulti Nal (Chandra Valley) 11600 ft Gramphu 12000 ft. Dhorni 12000 ft. Beas Valley near Marhi 11000 ft. Pir Panjal Range opposite Kulti Nal 12000 ft. Rhotang Pass 13000 ft.

13 *Nemoura rahlae* Jewett, *Proc Nat. Acad Sci.* Allahabad, 28 (B) 320 329
*Rhala 8835 ft.

14 *Rhabdiopteryx lunata* Kimmins Ann. Mag Nat. Hist., 13 (2) 721 740 (1946) Jewett *Proc Nat. Acad Sci* Allahabad 28 (B) : 320-329
Kulu Nal 11600 ft. Chhatru 11500 ft Hamta Jot 14500 ft. Kulu Valley Tibet Rongbuk 16500 ft. Everest Base Camp Rongbuk Glacier

Order TRICHOPTERA

FAMILY HYDROPSYCHIDAE

1 *Macronema punctatum* Betten RIM 3 232 (1909)
*Kulu

FAMILY CALAMOCERATIDAE

2 *Anisocerus fuscipennis* Albarda in Veth's Midden-Sumatra, 4 (5) 17 pl. v fig 1 Betten RIM 3 239 (1909)
*Kulu

FAMILY PHRYGANEIDAE

3 *Neuronia maclachlani* White, Proc. Ent. Soc London p 26 (1862) Betten, RIM 3 242 (1909)
*Kulu. Darjeeling Shillong
Genus is found only in Asia, two other species are known from Japan.

FAMILY PHILOPOTAMIDAE

4 *Dolophilodes tibetana* Kimmins Arkiv för Zoologi, 9 (2) 89 (1956)
*Kashmir Prang 5000 ft. W Tibet Valley of Digar Pola, 14500 ft.

FAMILY LIMNOPHILIDAE

5 *Platyphylax* sp McLachlan II Yarkand Miss. Trichoptera, p. 5 (1878)
*Leh
6. *Stenophylax micraulax* McLachlan II Yarkand Miss. Trichoptera, p. 5 (1878)
*Leh.

FAMILY RHYACOPHILIDAE

7 *Agapetus kashmirensis* Kimmins Arkiv For Zool. 6 (8) 176, fig 11 (1953)
*Kashmir Mamer
8 *Agapetus sindis* Kimmins Arkiv For Zool 6 (8) 175 fig 10 (1953)
*Kashmir Kangan R. Sind 5795 ft
9 *Glossoma moselyi* Kimmins Arkiv For Zool 6 (8) 172 fig 5 (1953)
*Kashmir Srinagar Harwan, R. Arrah.

Order NEUROPTERA

FAMILY ASCALAPHIDAE

1 *Ogcogaster segmentator* Westwood in Needham, RIM 3 198 (1909)
*Kulu Mandi
2. *Stylonotus obscurus* Westwood in Needham, RIM 3 199 (1909)
*Kulu Mandi

FAMILY MYRMELEONIDAE

3 *Formicaleo recandus* Walk. In Needham RIM 3 200 (1909)
*Kulu Kangra Valley Mussourie

4 *Macronemurus nefandus* Walk. in Needham, RIM 3 200 (1909)
*Kulu. Dehra Dun.

5. *Myrmeleon marginicollis* Gerst. in Needham RIM 3 200 (1909)
*Kulu.

6. *Myrmecaelurus punctulatus* Steven. in McLachlan II Yarkand Miss. Neuroptera p. 2 (1891)
*Leh. Hungary South Russia.

FAMILY CHRYSOPIDAE

7 *Ankylopteryx* ... Needham, RIM. 3 203 (1909)
*Kulu. Layallpur Pusa, Khasi Hills Sibsagar

FAMILY MANTISPIDAE

8. *Mantispa indica* Westwood in Needham RIM 3 195 (1909)
*Kangra Valley 4500 ft. Sikkim Upper Assam, Calcutta.

9 *Mantispa lineolata* Westwood in Needham RIM. 3 195 (1909)
Kulu

10 *Mantispa quadrituberculata* Westwood, In Needham RIM. 3 195 (1909)
Kulu Assam Sibsagar

FAMILY HEMEROBIIDAE

11 *Osmylus langi* McL. in Needham RIM 3 206 (1909)
*Kulu Kurseong

12. *Parosmylus pretiosus* Needham, RIM. 3 209 (1909)
*Kulu, Lahaul 10400 ft.

Order ORTHOPTERA

FAMILY ACRIDIDAE

1 *Sphingonotus fallax* Fieb. in Mistshenko Revision of Palaearctic species of the genus Sphingonotus Fieb (Orth Acrididae) Eos 1936.
*Kashmir

FAMILY LOCUSTIDAE

2. *Leptophyes angusticauda* Brunner Verh. Z.-b. Wien. 41 70 (1891)
*Kashmir

FAMILY GRYLLIDAE

3 *Gryllopsis terrestris* (Saus) In Chopard Ann Mag Nat. Hist. 16 (10) : 288 (1935)
Kashmir : Gandarbal 5200 ft.

4 *Gryllus conspersus* Walk. in Chopard Ann Mag Nat. Hist., 16 (10) 285 (1935)
*Kashmir Srinagar 5200 ft. Hazara Abbotabad 4000 ft.

5 *Liogryllus bimaculatus* (De Geer) in Chopard Ann. Mag Nat. Hist, 16 (10) 285 (1935)
*Kashmir Gandarbal 5200 ft Kodaikanal Palnis 7000 ft.

Order HETEROPTERA

FAMILY PENTATOMIDAE

1 *Acanthosoma forfex* Dall List Hem 1 308 (1851) Distant, FBI, Rhynchota, 1 317 (1902)
*Murree.

2 *Acanthosoma proximum* Dall List. Hem. 1 303 (1851) Distant II Yarkand Miss. Rhynchota, p 3 (1879) FBI., Rhynchota, 1 315 (1902)
*Murree. North India

3 *Bagrada picta* (Fab) Syst. Ent. p 715 (1775) (Cimex) Distant FBI Rhynchota 1 193 (1902)
*Murree. Hardwar Bengal Tirhoot, Calcutta, Manipur Bombay Ceylon Bagdad.

4 *Cydnus maurus* Dall List Hem 1 118 (1851) (Aethus) Distant, II Yarkand Miss Rhynchota p 3 (1879) FBI Rhynchota, 1 92 (1902).
*Jhelum Valley Throughout Hindostan Ceylon, Paradeniya.

5 *Dalpada confusa* Dist. II Yarkand Miss. Rhynchota p 4 (1879) FBI Rhynchota, 1 115 (1902)
*Murree.

6 *Dalpada tecta* Walk. in Distant, II Yarkand Miss. Rhynchota, p 4 (1879)
*Murree. Sylhet.

7 *Elasmostethus asperum* Walk. Cat Het. 2 395 (1867) Distant FBI, Rhynchota, 1 330 (1902)
*Murree. Sikkim.

8 *Elasmostethus recurvum* Dall List. Hem. 1 310 (1851) (Acanthosoma) Dist. FBI Rhynchota 1 328 (1902)
*Sind Valley

9 *Eurydema liturifera* (Walk.) Cat. Hem. 2 326 (1867) (Strachia) Mathew Agra Univ J Res (Sci.) (Suppl.) 4 749 (1955)
*Katrain. Sikkim Assam Khasi Hills, Naga Hills, China Sumatra, Burma

10 *Nezara viridula* (Linn.) Syst. Nat. (10) 1 444 (1758) Mathew Agra Univ J Res. (Sci) (Suppl.) 4 749 (1955)
*Manali 6500 ft. India Palaearctic, Nearctic Ethopian Neotropical, Oriental Australia

11 *Parastrachia exempta* (Walk.) Cat Het. 3 560 (1868) (Pravaca) Distant FBI Rhynchota 1 206 (1902)
*Murree. N Hindustan Naga Hills, Tenasserim.

12 *Phimodera rupshuensis* Hutchinson Mem. Conn Acad Arts & Sci. 10 120 (1934)
Indian Tibet Peldo-la near north end of Tso-Morari 14855 ft.

13 *Sastragala murreensis* Distant AMNH (7) 6 228 (1900) FBI Rhynchota, I 320 (1902)
*Murree.

14 *Sastragala rufispina* Distant, Trans ent. Soc. London p 353 (1887) FBI Rhynchota, I 319 (1902) Mathew Agra Univ J Res. (Sci) (Suppl) 4 750 (1955)
*Katrain 6000 ft. North India

15 *Urostylis femoralis* Walk. Cat. Het 2 413 (1867) Distant FBI Rhynchota I 307 (1902)
*Murree. Sikkim Mungphu Sylhet, Assam Burma Karennee.

Family Corixidae

16. *Camptopus lateralis* (Germ) in Distant, II Yarkand Miss Rhynchota (1879)
*Sind Valley Palaearctic range Europe Medeira Morocco, Astracan.

17 *Cletus punctulatus* (Westw) Hope. Cat 2 23 (1842) Distant, FBI Rhynchota 1 339 (1902) Mathew Agra Univ J Res. (Sci.) (Suppl.) 4 751 (1955)
Manali 6500 ft. Sikkim, Kurseong Khasi Hills Naga Hills.

18. *Elasmomia granulipes* (Westw) in Distant FBI Rhynchota, 1 392 (1902) Mathew Agra Univ J Res (Sci.) (Suppl.) 4 751 (1955)
*Manali 6500 ft. Sikkim.

19 *Stictopleurus* sp Hutchinson Mem. Conn Acad. Arts & Sci 10 121 (1934)
*Indian Tibet Tsak. Shang and Tsak-ra road from Tso-Morari to Tso-Kar 15000 ft.

Family Lygaeidae

20 *Arocatus pilosus* Dist., Trans Ent. Soc. London p. 123 (1879) FBI Rhynchota, 2 15 (1904)
*Murree.

21 *Bianchiella adelungi* Reuter in Hutchinson, Mem. Conn. Acad Arts & Sci., 10 128 (1934)
*Indian Tibet Igu in the Indus Valley above Leh, 11210 ft. Siberia Mongolia North China.

22. *Dalmacoris deserrans* Hutchinson Mem. Conn Acad Arts & Sci., 10 130 (1934)
*Indian Tibet Nying-ri & Chungang La 16800-17000 ft., under *Artemisia minor*

23 *Emblethis horvathiana* Hutchinson Mem Conn Acad. Arts & Sci 10 128 (1934)
Indian Tibet Renka la 16917 ft. between Mitpal Tso and Yaye Tso.

24 *Lamprodema brevicollis* Fiel. in Distant, II Yarkand Miss., Rhynchota, (1879)
*Tanktze to Chagra, Pangong Valley, Ladakh. Dalmatia (Europe

25 *Lygaeus pandurus* Scop Ent. Carn., p. 126 (1763) (*Cimex*) Distant, FBI, Rhynchota, 2 6 (1904) (*militaris*) FBI., Rhynchota, 6 4 (191
*Murree. Hardwar Bombay Bengalore, Mysore, Burma, Minhala, Malayan Archepalago, Australia, S Africa.

26. *Microplax kussarensis* Kritshanko in Hutchinson, Mem. Conn. Acad. Arts & Sci., 10 128 (1934)
*Indian Tibet Between Tsak-shang & Tsak-ra road from Tso Morari to Tso-Kar 15000 ft. North Bukhara.

27 *Nysius ericae* (Schill.) in Hutchinson, Mem. Conn. Acad. Arts & Sci, 10 122 (1934)
*Leh. Tsak-shang north of Tso-Morari, Kayann-la Koh Lungpa Valley Renka la between Mitpal Tso & Naye tso and Ororotse Tso. Central Asia.

28 *Nysius ericae alticola* Hutchinson Mem. Conn. Acad., Arts & Sci, 10 126 (1934)
*Indian Tibet Ororotse Tso 17381 ft. Kyang La, 16700-17000 ft.

FAMILY ARADIDAE

29 *Brachyrhynchus tagalicus* (Stal.) Of. Vet. Ak. Forh., p. 672 (18?) FBI, Rhynchota, 1 392 (1902) Mathew Agra Univ J Res. (Sci.) (Suppl) 4 751 (1955)
*Pir Panjal Range Chandra Valley near Gramphu, 12000 ft. Burma, Philippines, Java.

FAMILY PYRRHOCORIDAE

30 *Physopelta gutta* (Burm.) Nov Act. Acad. Leop., 16, (Suppl.) 300 (183 Distant, FBI Rhynchota 2 97 (1903) Mathew Agra Univ J. Res (Sci.) (Suppl.) 4 752 (1955)
Dalhousie. Assam, Khasi Hills Ceylon, Burma, Japan, Sumatra.

FAMILY REDUVIIDAE

31 *Acanthaspis apicata* Distant AMNH 7 (11) 354 (1903) FBI., Rhynchota, 2 266 (1904)
Kashmir Ootacamand.

32. *Harpactor iracundus* (Scop) in Distant, II Yarkand Miss Rhynchota (1879) FBI Rhynchota, 2 332 (1904) (*Reduvius*)
*Sind Valley European form.

33 *Harpactor nuceri* (Distant) II Yarkand Miss. Rhynchota, p. 11 (18 FBI Rhynchota 2 335 (1904) (*Harpactor*)
Sind Valley Kashmir Baltistan. Sylhet.

34 *L* ... *ora* Stal. Of. Vet Atk. Forth p 192 (1859) FBI Rhynchota

2:238 (1903) Mathew Agra Univ J Res. (Sci) (Suppl) 4 ,52 (1955)
*Manali. Burma Java.

35 *Pirates affinis* (Serv) Ann. Sc. Nat. 23:216 (1831) (*Pirates*) Distant, FBI. Rhynchota 2·299 (1904)
*Jhelum Valley Khasi Hills Bombay Burma Rangoon Teinzo Bhamo Malayan Peninsula, Cochinchina Java Borneo

36. *Pirates flavipes* (Walk.) Cat. Het. 7·93 (1873) (*Lestomerus*) Distant FBI Rhynchota, 2:297 (1904)
*Kangra Valley Berhampur (Bengal)

37 *Sphedanolestes funeralis* Distant, AMNH 7 (11) 210 (1903) FBI Rhynchota 2.340 (1904)
*Kashmir

FAMILY ANTHOCORIDAE

38. *Anthocoris gyalpo* Hutchinson Mem Conn. Acad Arts & Sci. 10 136 (1934)
*Indian Tibet Leh. Apparently blown from *Populus* sp

39 *Ectemnus paradoxus* Hutchinson Mem. Conn Acad. Arts & Sci. 10 134 (1934)
*Indian Tibet Igu in the Indus Valley above Leh, on the bark of *Populus* sp., 11210 ft

FAMILY MIRIDAE

40. *Chlamydatus pachycerus* Kiritsh. in Hutchinson Mem. Conn. Acad. Arts & Sci., 10 140 (1934)
Indian Tibet Shakya La, 17000 ft. Kyang La 16800-17500 ft. Ororotse Tso 17381 ft. Marsimik La 17400 ft. Kyam 15550 ft. Nyagtzu 15324 ft., Peldo-le north end of Tso-Morari 14835 ft. and Taak-shang Southern Tibet 3rd Mt. Everest Expedition, 15500-16500 ft.

41 *Dicyphus physochlaenae* Hutchinson Mem. Conn. Acad. Arts & Sci., 10 138 (1934)
*Indian Tibet Dambguru 15100 ft on *Physochlaena praealta* Hook. (Solenaceae)

42. *Dicyphus seiggs* Hutchinson Mem. Conn. Acad. Arts & Sci. 10 139 (1934)
Indian Tibet between Tangtse and Mugleb, 4175 m.

43 *Tibetocoris margaretae* Hutchinson, Mem. Conn. Acad Arts & Sci. 10 14? (1934)
*Indian Tibet Chang-chenmo River near Pamzal 17000-17300 ft. Nyingri 16800 ft. Chungang La 17400 ft. Kakstet La 17600 ft

FAMILY CAPSIDAE

44 *Calocoris lineolatus* (Goeze) Ent. Beytr 2:267 (1878) (*Cimex*) Distant, FBI., Rhynchota 2·451 (1904)

24 *Lamprodema brevicollis* Fiel in Distant II Yarkand Miss., Rhynchota (1879)
*Tanktze to Chagra, Pangong Valley Ladakh. Dalmatia (Europe).

25 *Lygaeus pandurus* Scop., Ent. Carn p 126 (1763) (*Cimex*) Distant, FBI, Rhynchota, 2 6 (1904) (*militaris*) FBI Rhynchota, 6 4 (1916).
*Murree. Hardwar Bombay Bengalore, Mysore, Burma, Mandey, Minhala, Malayan Archepalago Australia, S Africa.

26 *Microplax kusarensis* Kritshanko in Hutchinson Mem. Conn. Acad. Arts & Sci. 10 128 (1934)
*Indian Tibet Between Tsak shang & Tsak-ra, road from Tso-Morari to Tso-Kar 15000 ft North Bukhara.

27 *Nysius ericae* (Schill.) in Hutchinson, Mem. Conn. Acad. Arts & Sci, 10 122 (1934)
*Leh. Tsak shang north of Tso-Morari Kayann-la Koh Lungpa Valley, Renka la between Mitpal Tso & Naye tso and Ororotse Tso. Central Asia.

28 *Nysius ericae alticola* Hutchinson Mem Conn Acad., Arts & Sci., 10 126 (1934)
*Indian Tibet Ororotse Tso 17381 ft Kyang La, 16700-17000ft.

FAMILY ARADIDAE

29 *Brachyrhynchus tagalicus* (Stal.) Ofv Vet. Ak. Forh. p. 672 (187) FBI, Rhynchota 1 392 (1902) Mathew Agra Univ J Res. (Sci.) (Suppl) 4 751 (1955)
*Pir Panjal Range Chandra Valley near Gramphu, 12000 ft. Burma, Philippines Java.

FAMILY PYRRHOCORIDAE

30 *Physopelta gutta* (Burm) Nov Act Acad. Leip. 16 (Suppl.) 300 (183? Distant, FBI Rhynchota 2 97 (1903) Mathew Agra Univ J. Res (Sci.) (Suppl) 4 752 (1955)
*Dalhousie. Assam, Khasi Hills Ceylon Burma Japan, Sumatra.

FAMILY REDUVIIDAE

31 *Acanthaspis apicata* Distant AMNH 7 (11) 354 (1903) FBI Rhynchota 2 266 (1904)
*Kashmir Ootacamand.

32 *Harpactor iracundus* (Scop) In Distant II Yarkand Miss., Rhynchota (1879) FBI Rhynchota 2 332 (1901) (*Reduvius*)
*Sind Valley European form.

33 *Harpactor reuteri* (Distant) II Yarkand Miss. Rhynchota p. 11 (18 FBI Rhynchota 2 335 (1901) (*Harpiscus*)
*Sind Valley Kashmir Baltistan Sylhet

34 *Lisarda rhypara* Stal Ofv Vet Atk. Forth p 192 (1859) FBI Rhynchota

6 *Cicadula indica* Pruthi Mem Indian Mus 11.54 p l iv figs 7 7a (1930) Murree Hills Tret, Kohala Dalhousie Hills. Calcutta Nerbuda Valley (C. P) Salem Distt. (S India)

7 *Cicadula indrica* Pruthi Mem Indian Mus 11·61 pl v fig 5 (1930) *Chamba.

8 *Cicadula montanus macropterus* Pruthi Mem. Indian Mus 11.58 pl iv fig 9 pl v fig 1 1a (1930) *N W Himalaya Murree Hills Dayankund Dalhousie Hills

9 *Deltocephalus pepn* Pruthi, Mem. Indian Mus 11 127 pl. ix fig 9 (1936) *Kuldanna, Murree Hills

10 *Durgades indiocerus* Pruthi Mem. Indian Mus 11 13 (1930) *Murree Hills 4-7000 ft.

11 *Eurinthus extremus* (Walk.) List. Hom. 3 701 (1851) (*Tettigonia*) Distant FBI Rhynchota 4:227 (1908) *Murree. North India.

12. *Eugnathodus indica* Pruthi, Mem Indian Mus 11 48 pl iv fig 4 4a 4b (1930) Kohala Tret (Murree Hills) Layallpur Nerbuda Valley Koilari Harra, Santal (C. P) North Malabar

13 *Eugnathodus* (*Nesosteles*) *sanguinescens* (Kirk.) Pruthi, Mem. Indian Mus. 11.52 pl. iv figs.6 6a, (1930) Murree Hills Layallpur Koilari Sarrai. Amarkantak, Nerbuda Valley Calcutta.

14 *Gurna minoricephala* Pruthi, Mem Indian Mus 11:29 pl.ii figs 10a, 10b (1930) *Jhika Gali Murree Hills

15. *Krisna strigicollis* (Spin.) Mem di Matem e di Fis Soc Ital Modena, p 167 (1852) (*Siva*) Distant, FBI Rhynchota, 4:297 (1908) *Kangra Valley Mussoorie, Kanti Pusa Dacca Raniganj Mamari Calcutta Nilgiri Hills Bombay Burma Ruby Mines Tenasserim Malayan Peninsula Perak Cambodia Java Borneo, Celebes Sangir Philippines North China

16 *Ledra mutica* Fabr Syst. Rhynch. p 25 (1803) Distant FBI Rhynchota 4 173 (1908) Kangra Valley Mussoorie, Pusa Calcutta, Bombay Burma Pegu

17 *Ophiola bicolor* Pruthi Mem Indian Mus 11 123 pl. ix, figs 6 6a (1936) *Dayankund Nullah (Dalhousie) Dalhousie Khajiar Road, 8000 ft. Bakrota Hill.

18. *Thamnotettix chhota* Pruthi Mem. Indian Mus. 11·64 (1930) *Chamba Pashok (Darjeeling Distt.)

FAMILY FULGORIDAE

19 *Proutia confusa* Distant FBI Rhynchota, 3.386 (1906) *Sind Valley

FAMILY CICADIDAE

20 *Paharia casyapae* (Distant) AMNH (6) 1 374 (1888) (*Tibicen*) FBI Rhynchota, 3 163 (1906)
*Kashmir Valley

FAMILY CERCOPIDAE

21 *Poophilus costalis* (Walk.) List Hom. 3 707 (1851) (*Ptyelus*) Distant, FBI, Rhynchota, 3 86 (1908)
*Dras Kargil, Leh Dacca, Pusa Bombay Surat, Calcutta, Bangalore, Darjeeling Karachi Ceylon Paradeniya, Nalanda, Anuradhapura, Singapore, S & W Africa, Natal Nyasaland

22 *Ptyelus nebulosus* (Fab) Syst. Ent. 4.50 (1794) (*Cercopis*) Distant, FBI Rhynchota, 4 88 (1908)
*Sind Valley North Bengal Jamalpur Calcutta Bombay Dacca Bangalore Ceylon Ephawela.

Order COLEOPTERA

FAMILY CARABIDAE

1 *Agonum ladakense* Bates in Jadlicka Hundukush Expedition, Arb. morph. taxon. Ent. Berlin-Dahlem, 4 (3) 189 (1937)
*Kashmir Pamir Tibet, Jalalabad.

2 *Amara bruci* Andrewes Ann Mag Nat. Hist. (9) 11 176 (1923) Mem. Conn Acad. Arts & Sci. 10.25 (1934)
*Indian Tibet Ororotse-tso 17400 ft. Anem la 17000 ft. Tso-Nyak repoc 14300 ft. Second Mount Everest Expedition Base Camp.

3 *Amara trivialis* Gyll in Bates Second Yarkand Miss., Coleoptera, (1891).
*Sind Valley West Europe

4 *Anchomenus politissimus* Bates, II Yarkand Miss., Coleoptera, (1891).
*Murree.

5 *Anthia orientalis* Hope, in Bates II Yarkand Miss. Coleop. (1891).
*Jhelum Valley

6 *Bradytus apricarius* (Payk.) in Bates II Yarkand Miss. (1891)
*Sind Valley Dras Kargil Leh. Pamir between Sirikol and Panja

7 *Callistomimus chalcocephalus* (Weid.) in Bates, II Yarkand Miss., Coleoptera (1891) (*Pristomachaerus*)
*Jhelum Valley Hong Kong

8. *Cymindis altica* Gebl in Bates II Yarkand Miss Coleop., (1891).
*Between Dras and Leh.

9 *Cymindis championi* Andrewes Ann Mag Nat Hist. (10) 2.589 (1928) Mem Conn. Acad Arts & Sci 10.25 (1934)
*Tibet Tso-Nyak, 14300 ft. Northern Kumaon and Tibet.

10 *Cymindis rubriceps* Andrewes, Mem. Conn. Acad. Arts & Sci 10.25 (1934)
*Indian Tibet Anem La 17000 ft.

11 *Elkra cometes* Andrewes Ann Mag Nat. Hist 10 (18) 63 (1936)
Kashmir

12. *Harpalus japonicus* Morawitz In Bates II Yarkand Miss. Coleoptera (1891)
*Murree. China Japan, Formosa.

13 *Harpalus quadricollis* Koll & Redt. in Bates, II Yarkand Miss Coleopetera (1891)
*Dras Leh.

14 *Hypolithus perlucens* Bates II Yarkand Miss Coleoptera (1891)
Jhelum Valley

15 *Sphodrus cordicollis* Chaud n Bates II Yarkand Miss Coleoptera (1891
*Murree.

16. *Melops piliferus* Bates II Yarkand Miss Coleopetera (1891)
*Murree.

17 *Scarites mesus piceus* Chaud in Bates, II Yarkand Miss. Coleoptera, (1891) Andrewes FBI Carab 1:253 (1929)
*Jhelum Valley Nepal Assam Sibsagar Sikkim Bengal Calcutta Bihar Chapra Muzaffarpur U P Ranikhet Nainital Dehra Dun N W F P Kohat Indore C. P Nagpur Madras Yarcaud

18. *Calathus rufescens* Andrewes Keys to some Indian genera of Carabidae (Col) IV Genus *Calathus* *Stylops* 3·217 (1934) Lindroth Trans. R. ent. Soc London, 108 (11) 558 (1956)
*Kashmir Pahalgam

19 *Pristena braccata* Andrewes, Keys to some Indian genera of Carabidae (Col) IV Genus (*Calathus* *Stylop* 3.211 (1934) Lindroth, Trans R. ent. Soc. London 108 (11) 549 (1956)
*Kashmir Pahalgam

20 *Pristena chambae* Andrewes Keys to some genera of Carabidae (Col) IV Genus *Calathus* *Stylops* 3:215 (1934) Lindroth Trans R ent. Soc. London 108 (11) 549 (1956)
*Chamba.

21 *Pristena glacialis* (Andrewes) Keys to some Indian Genera of Carabidae (Col.) IV The Genus *Calathus* *Stylop* 3 218-219 (1934) (*raptor*) Lindroth Trans. R. ent. Soc. London 108 (11) 554 (1956)
Kashmir

22 *Pristena laevops* Andrewes Keys to some genera of Indian genera of Carabidae (Col.) IV The Genus *Calathus* St lops 3:211 (1934) Lindroth Trans. R. ent. Soc. London 108 (11) 549 (1956)
*Kashmir Uri Pahalgam.

23 *Pristena macra* Andrewes Keys to some Indian genera of Carabidae (Col.) IV Genus *Calathus* *Stylops* 3·212 (1934) Lindroth Trans R. ent. Soc. London, 108 (11) 549 (1956)
*Kashmir

FAMILY DYTISCIDAE

24 *Agabus* (*Anagabus*) *jucundus* Guignot Opusc. ent. Lund, 19 221 224 (1954)
Kashmir Karg'l

25 *Agabus* (*Dichonectes*) *nitidus* F in Guignot Opusc. ent Lund, 19:221-224 (1954)
*Kashmir Sonemarg Dras, Kargil Ladakh Nima Digar Polu, Baoshaple.

26 *Agabus* (*Gaurodytes*) *adustus* Guignot, Opusc ent. Lund 19 221 224 (1954)
*Kashmir Chusol (Ladakh) 4336 m

27 *Coelambus confluens* F in Guignot Opusc Ent Lund 19 221 224 (1954)
*Kashmir Pampur

28 *Coelambus flaviventris* Motsch in Guignot Opusc. ent Lund 19:221 224 (1954)
*Ladakh Thongmon Tso

29 *Colymetes lineatus* Koll & Redt in Hügel's Kashmir p 502 (1848)
*Kashmir

30 *Cybister lateralimarginalis lepidus* Apetz in Guignot, Opusc. ent Lund, 19-221 224 (1954)
*Kashmir Shadipur Bond Lokut, Dal Lake Pharla Kuri, Gangribal, Nishat Bagh.

31 *Cybister tripunctatus asiaticus* Sharp in Guignot Opusc ent. *Lund 19:221-224* (1954)
Kashmir Srinagar Naopur Ladakh Nishat Bagh Chushol.

32 *Guignotus pusillus geminus* F in Guignot Opusc. ent Lund 19.221-224 (1954)
*Kashmir Pampur Gangribal Galam Pur Nishat Bagh Sumbal & Wular Lakes Ladakh Kargil [Punjab Sohana]

33 *Hydrovatus cordaticollis* Reitt. in Guignot Opusc ent Lund 19:221-224 (1954)
*Kashmir Shimalia Karbu

34 *Noterus clavicornis convexiusculus* Reiche in Guignot Opusc. ent. *Lund*, 19-221 224 (1954)
Kashmir Gangribal Jhil opposite Hajan Srinagar Lokut Dal [Punjab Sohana]

35 *Potamonectes* (*s str*) *griseostriatus* Deg in Guignot Opusc *ent Lund*, 19 221 224 (1954)
*Kashmir Ladakh Digar Polu Shargola, Kangral Spring below Fotu La Chusol Tso Kar 4527 m Spitok Galam Bagh.

36 *Rhantus pulverosus* Steph. in Guignot Opusc ent Lund 19.221 224 (1954)
*Ladakh Kashmir Sonamarg Kangan Ghulam Bagh, Srinagar

37 *Trochalus rugulosus* Koll. & Redt in Hügel's *Kaschmir.*, p. 503 (1848)
Kashmir

FAMILY HALIPLIDAE

38. *Haliplus maculi*... um ... Yarkand Miss. Coleoptera
p 37 (1891)
*Jhelum Vall...

FAMILY HYDROPHILIDAE

39 *Berosus (s str) signaticollis* (Charpentier) in d Orchymont Bull. Mus roy Hist. nat. Belg 14 (60) 4 (1943)
*Wular Lake near Kuhmus 1573 m.

40 *Berosus (Enoplurus) indicus* Motschlsky in d Orchymont, Bull. Mus roy Hist. nat. Belg 19 (60) 4 (1943)
*Phasha Kuri near Pampur 1585 m.

41 *Coelostoma* sp Pruthi Indian J Ent., 1 (1) 66 (1939)
Manali, 6000 ft.

42. *Enochrus (Lumetus) halophilus* (Bedel) in d Orchymont Bull. Mus roy Hist. nat. Belg 19 (60) 3 (1943)
Srinagar 1585 m. Gangribal 1580 m. Phasha Kuri near Pampur 1585 m. Wular Lake Kuhmus 1573 m Jhil of Bakh Hajan near Wular 1575 m. Jhil south-east of Hajan 1580 m.

43. *Helophorus (s str) ? griseus* Herbst in d Orchymont Bull Mus roy Hist. nat. Belg 19 (60) 3 (1943)
Phasha Kuri near Pampur 1585 m.

44 *Helophorus (s str) splendidus simlaensis* d Orchymont Bull Mus roy Hist. nat. Belg., 19 (57) 11 1943)
Ladakh Bao 4616 m. Kyam Valley of River Chang Chenmo 4725 m. Sta-rtsk-puk-tso (Tso Bar) 4538 m C. Tibet Phari or Pari Kampa Dzhong 14500 ft. (4420 m) Lingka 420 m. Tingri 15000 ft. (4572 m.)

45 *Helophorus (Atracthelophorus) frater* d Orchymont, Bull Mus. roy Hist. nat. Belg 19 (57) 11 (1939)
Ladakh Tso-Morari 15000 ft. Frontier of Tibet and Kumaon Laptel 15000 ft. (4572 m) Sangchar 14500 ft. (4420 m.)

46 *Helophorus (? Atracthelophorus) montanus* d Orchymont, Bull Mus roy Hist. Nat. Belg 19 (57) 11 (1943)
*Ladakh Fotu la 3720 m. W Tibet Sulphur spring south of lake Mang zaka 5400 m. C. Tibet Tingri 15000 ft., Lingka and Shekkar 14500 ft. Gyangtse 15000 ft (3962 m.) Tibet Kumaon Frontier Laptel 15000 ft. Outside Tibet Aulie Ata en Syr Darya.

47 *Helophorus lentus* Sharp In Pruthi Indian J Ent. 1 (1) 66 (1939)
Manali, Hot spring 6000 ft. Ceylon Indo-China South Asia and Nepal.

48. *Helophorus (Lihelophorus) orr* Zaitzeva, in d Orchymont, Bull Mus. roy Hist. nat. Belg 19 (57) 9 (1943)
Ladakh Kyam in the Valley of River Chang Chenmo 4725 m. Cuthol south of Pangong tso 4336 m. W Tibet 50 klm. east of Pangong C. Tibet 4863 m. East Tibet Region of Kuku-Nor Mang Tso 14500 ft.

49 *Helophorus (Meghelophorus) aquaticus* Linn. in d Orchymont Bull. Mus. roy Hist. nat. Belg 19 (57) 10 (1943)
Ladakh East of Mugleb between Mugleb and Tangtse 4175 m. W Tibet North of Pangong Lake

50 *Hydrophilus caraboides* (Linn.) in d Orchymont Bull. Mus. roy Hist. nat. Belg., 19 (60) 4 (1943)

*East of Sonamarg 2620 m. Sonamarg 2620 m.

51 *Laccobius (s str) kingstoni* d Orchymont, Bull. Mus. roy Hist. nat. Belg 19 (57) 12 (1943)
*Ladakh Cushol south of Pangong Tso 4336 m. C. Tibet. Kampa Dzhong 10500 ft. Tingri 15000 ft. (4572 m.)

52 *Laccobius (s str) kashmirensis* d'Orchymont, Bull. Mus. roy Hist, nat. Belg 19 (60) 1 12 (1943)
*Kashmir Valley 5-6000 ft. Gangribal 1580 m., Kruhmus 1573 m.

53 *Sttharus (s str) piceus* (Linn.) in d Orchymont, Bull. Mus. roy Hist. nat. Belg 19 (60) 4 (1943)
*Srinagar east of rout to Gangribal 1580 m. Dal lake near Nishat Bagh *1580* m. *Jhil* southeast of Hajan *1580* m. *Bod-Dal south of Hazratbal* 1582 m

Family Staphylinidae

54 *Aleochara (Coprochara) bilineata* Gyll. in Cameron Mem. Conn. Acad. Arts & Sci. 10.21 (1934)
*Indian Tibet Tsak-shang above Tso-Morari 15985 ft.

55 *Atheta (Alocenota) iguensis* Cameron Mem. Conn. Acad. Arts & Sci., 10.19 (1934)
*Indian Tibet Igu 11210 ft.

56 *Atheta (Dimetrota) hutchinsoni* Cameron Mem Conn. Acad. Arts & Sci., 10 ?1 (1934)
*Indian Tibet Marsimik La 18394 ft. Ororotse 17381 ft.

57 *Geodromicus similis* Cameron FBI Staphylinidae, 1 455
*Kashmir Gulmarg Feroxpur Nullah, 5500 ft.

58. *Geodromicus affinis* Cameron Mem Conn Acad Arts & Sci. *10 17 (1934)*
*Indian Tibet Kargil

59 *Lesteva kargilensis* Cameron, Mem. Conn Acad Arts & Sci *10 17 (1934)*.
*Indian Tibet Kargil

60 *Paederus fuscipes* Curt II Yarkand Miss. *Coleoptera (1891)*
*Murree.

61 *Philonthus cyanelytrus* Kr in Sharp II Yarkand Miss. *Coleoptera, (18?)*
Cameron FBI Staphylinidae 3 86 (1932)
*Murree. Simla Hills 7-8000 ft.

62. *Stenus caporiaccoi* Bernhauer Atti Mus Stor nat. Trieste *12 06 (1935)*
*Kashmir

63 *Stilicus caporiaccoi* Bernhauer Atti Mus. Stor nat. Trieste, *12:87 (1935)*
*Kashmir

Family Cantharidae

64 *Cantharis antennalis* Bates II Yarkand Miss. Coleoptera *(1891)*
*Sind Valley

65 *Cantharis bicellata* Fairm, C. R. Ent. Belg 35 130 *(1891)* Him. Is.?
Expedition Results Arb morph taxon. Ent. Berlin Dalhem, 4(3) 181 ?

*Kashmir Karakoram Tibet, North India.

66. *Catharus dissipennis* Fairm C. R. Ent. Belg 35 100 (1891)
*Kashmir

67 *Catharus fasciangulata* Fairm, C. R Ent. Belg 35 132 (1891)
Kashmir

68. *Epicauta quadraticollis* Fairm C. R Ent. Belg 35 101 (1891)
*Kashmir

69 *Epicauta hoagei* Bates II Yarkand Miss Coleoptera p. 28 (1891)
*Murree.

70. *Zonitis nigripectus* Fairm C. R. Belg 35 133 (1891)
*Kashmir

FAMILY RHIPIDOCERIDAE

71 *Lichas giganteus* Fairm. C. R Ent. Belg 35 128 129 (1891)
*Kashmir

72. *Lichas trapezicollis* Fairm. C. R Ent. Belg 35 129 (1891)
*Kashmir

FAMILY ELATERIDAE

73 *Campsolacon aequalis* Cand. in Fleutiaux, Hindukush Expedition Arb. morph taxon Ent. Berlin-Dalhem, 3 (3) 180 (1936)
*Kashmir 11000 ft. Wama Afghanistan.

74 *Diacanthus amplatus* Fairm. C. R Ent. Belg 35 127 (1891)
Kashmir

75 *Diacanthus picticollis* Fairm. C. R. Ent. Belg 35 128 (1891)
Kashmir

76. *Diacanthus sendaeraeneus* Fairm. C. R. Ent. Belg 35 128 (1891)
*Kashmir

FAMILY BUPRESTIDAE

77 *Ancylocheria costipennis* Fairm. C. R Ent. Belg 35 126 (1891)
*Kashmir

FAMILY CISTELIDAE

78 *Allecula (Dietopsis) costipennis* Bates, II Yarkand Miss. Coleopetra p 76 (1891)
Murree.

FAMILY LAGARIIDAE

79 *Lagria indicola* Bates II Yarkand Miss. Coleoptera, p 77 (1891)
Murree.

FAMILY PEDILIDAE

80. *Pyrochroa subcostulata* Fairm. C. R Ent. Belg 35 102 (1891)
*Kashmir

FAMILY ODEMERIDAE

81 *Chrysanthia fuscimembris* Fairm. C R. Ent. Belg 35 134 (1891).
*Kashmir

82 *Salenomus nebulosus* Fairm C R Ent Belg 35 133 (1891)
*Kashmir

FAMILY COCCINELLIDAE

83 *Chilocorus bijugus infernalis* Muls. Ann Co Linn. Lyon (2) 1 189 (1855)
Kapur RIM 52 (2-4) 259 (1956)
*Kashmir Tangmarg 7-8000 ft. Srinagar 5000 ft. Almora 5500 ft. (Kumaon Hills) Shillong 4900 ft.

84 *Chilocorus rubidus* Hope, in Gray Zool Misc. 31 (1831) Kapur RIM. 52 (2-4) 262 (1956)
*Kashmir Srinagar 5500 ft. Almora 5500 ft Chitral Izha Malna Penang Celebes Australia Nepal China Mongolia Manchuria, Japan USSR.

85 *Epilachna ocellata* Redt Hugel s Kaschmir 4(2) 497 564 (1844)
Kapur RIM 48 18 (1950)
*Kashmir Gharial Murree Hills 6000 ft Naggar to Manali. Simla Hills 7-8000 ft. Phagu 9000 ft Kufri 7000 ft Almora (Kumaon Hills) Nepal Sikkim E. Himalaya Mungphu (Darjeeling)

86 *Halyzia tschitscherini* Sem. in Korschefsky Hindukush Expedition, Arh. morph taxon Ent Berlin Dalhem, 4(3) 183 (1937)
*Srinagar Kashmir Chitral Karakel Bumboret Valley Sikkim

87 *Leis dimidiata* (Fab) Species Insectorum 94 (1781) (*Coccinella*) Kapur RIM 52 (2-4) 332 (1954)
*Kashmir Kulu Palampur Gurdaspur (Punjab) Sikkim, Nepal, Assam, Bengal (Calcutta) Manipur China, Japan.

88 *Pharoscymnus flexiblis kashmirensis* Kapur R. I M 52 (2-4) 267 (1956).
*Kashmir Srinagar 5500 ft West Almora 5500 ft Cherat 4000 ft, N W F P

89 *Sticholotis marginalis* Kapur R I M. 52 (2-4) 270 (1956)
*Kashmir Srinagar

FAMILY MELOIDAE

90 *Meloe semicoriaceus* Fairm C. R. Ent Belg 35 102 (1891)
*Kashmir

91 *Meloe transversicollis* Fairm C. R Ent. Belg 35 102 (1891)
*Kashmir

92. *Mylabris cichorii* (L.) Museum Ludvicae ulricae Regina, 103 (1764)
(*Meloe*) Kapur RIM 52 (2-4) 329 (1954)
*Murree Gilgit. Dehra Dun Lucknow Calcutta Darjeeling Dist., Sikkim Mysore and Manipur

93 *Mylabris macilenta* Marseul in Bates II Yarkand Miss Coleoptera p 28 (1891)
*Murree.

94 *Mylabris sidae* Fab in Bates II Yarkand Miss Coleoptera p 78 (1891)
*Sind Valley Murree.

Family Malachiidae

95 *Malachius coerulleostulatus* Fairm C R. Ent Belg p 130 (1891 Hadersdorf-Weidlingan Hindukush Expedition Arb. morph taxon Ent. Berlin-Dahlem, 4(3) 181 (1937)
Kashmir Afghanistan Ptsigulu tal

Family Bostrichidae

96. *Heterobostrychus aequalis* (Waterhouse) Proc. Zool Soc London 215 (1884) (*Bostrichus*) Kapur RIM 52 (2-4) 336 (1954)
*Kashmir Malabar Bengal Assam Burma, New Guinea and Madagascar

Family Tenebrionidae

97 *Ascelosodis assimilis* Bates Second Yarkand Mission, Coleop p 57 (1891)
Dras Kargil Leh

98. *Ascelosodis ciliatus* Bates Second Yarkand Mission Coleop p 57 (1891)
*Dras Kargil Leh.

99 *Ascelosodis grandis* Bates Second Yarkand Mission Coleop p 58 (1891)
Dras Kargil, Leh.

100 *Ascelosodis inermis* Fairm. C R. Ent. Belg 35 93 (1891)
Kashmir

101 *Ascelosodis intermedius* Bates Second Yarkand Mission, Coleop p 58 (1891)
*Dras Kargil Leh

102. *Ascelosodis scandens* Fairm. C. R. Ent. Belg 35 93 (1891)
*Kashmir

103. *Blapidurus crassicornis* Fairm. C. R Ent. Belg 35 96 (1891)
*Kashmir

104 *Blapidurus marginicollis* Fairm C R. Ent Belg 35 131 (1891)
*Kashmir

105 *Bioramix indiodes* Bates Second Yarkand Mission Coleop p 71 (1891)
*Sind Valley

106 *Bioramix ovalis* Bates, Second Yarkand Mission p 71 (1891)
*Dras, Kargil Leh.

107 *Bioramix puncticeps* Bates Second Yarkand Mission Coleop p 71 (1891)
Dras Kargil, Leh.

108. *Blaps indicola* Bates Second Yarkand Mission, Coleop p 61 (1891)
Sind Valley

109 *Blaps ladakensis* Bates Second Yarkand Mission, Coleop. 62 (1891).
*Yanktze to Chagra Pangong Valley

110 *Blaps lucens* Fairm C. R. Ent. Belg 35 95 (1891)
*Kashmir

111 *Blaps perlonga* Bates Second Yarkand Mission, Coleop p 62 (1891)
*Yanktse to Chagra Pangong Valley

112 *Blaps urophora* Fairm C R. Ent. Belg 35 95 (1891)
*Kashmir

113 *Botiras punctatellus* Fairm C. R Ent. Belg 35 99 (1891)
*Kashmir

114 *Botiras sculptipennis* Fairm. C R. Ent. Belg 35 99 (1891)
*Kashmir

115 *Botiras striatellus* Fairm C. R. Ent Belg 35 98 (1891)
*Kashmir

116 *Capnisa mediocris* Fairm. C R. Ent. Belg 35 93 (1891)
*Kashmir

117 *Chianalus costipennis* Bates Second Yarkand Mission, Coleop. p. 72 (1891).
*Dras Kargil. Leh.

118 *Chianalus subcostipennis* Grid. Att. Mus Stor nat Trieste, 12 64 (1935).
*Kashmir

119 *Coelocnemodes stoliczkanus* Bates Second Yarkand Mission, Coleop. p. 65 (1891)
*Murree.

120 *Cyphogenia depressiuscula* Fairm C. R. Ent. Belg 35 92 (1891)
*Kashmir

121 *Cyphogenia plana* Bates, Second Yarkand Mission, Coleop. p. 60 (1891).
*Dras, Kargil Leh Pangong Valley

122. *Dichillus denoi* Grid. Att. Mus. Stor nat. Trieste 12 58 (1935).
*Kashmir

123 *Faustia laevuscula* Fairm. C. R. Ent Belg 35 97 (1891)
*Kashmir

124 *Gauromaia tenuestriata* Fairm. C. R Ent. Belg 35 132 (1891)
*Kashmir

125 *Gonocephalum (s str) guerrei* Chatan. Bull Mus. Nat. Paris, p 241 (1917).
*Himalaya. Burma, China Afghanistan Wama.

126 *Gonocephalum (s str) simulatrix* (Fairm) in Schuster Arbeit. morphol taxon Entom Berlin-Dahlem 3 193 (1936)
*Himalaya Afghanistan.

127 *Gonocephalum (s.str) tuberculatum* Hope in Kaszab, Entom. Arbeit. Mus G Frey Munich pp 431 464 (1936)
*Himalaya India Burma Annam China Formosa.

128 *Laena (Catolaena) similima* Schuster Ann Mag Nat Hist. 16 (10) 462 (1935)
*Gulmarg

129 *Lacna cribrella* Rett. in Schster Ann. Mag Nat. Hist. 16 (10) 438 (1935)
*Batote 5800 ft. Udhampur Dis (Kashmir)

130. *Lacna carinipennis* Schuster Ann. Mag Nat. Hist. 16 (10) : 448 (1935)
Kulu.

131 *Lacna lacordairei* Marseul in Bates Second Yarkand Miss Coleoptera p. 76 (1891)
Sind Valley

132 *Lacna nigritissima* Rett. in Schuster Ann Mag Nat. Hist. 16 (10) 438 (1935)
*Gulmarg 8500 ft. Jhelum Valley Ferozpur Nullah

133 *Lacna nitida* Schuster Ann. Mag Nat Hist. 16 (10) 453 (1935)
*Gulmarg 8500 ft. Jhelum Valley

134 *Lacna punctiventris* in Schuster Ann. Mag Nat. Hist. 16 (10) 438 (1935)
Gulmarg 8500 ft. Jhelum Valley Bushahr State 6000-8000 ft.

135. *Lacna lacordairei* Marseul, in Bates Second Yarkand Mission Coleop p. 76 (1891)
Sind Valley

136. *Leptomorpha brevicollis* Fairm. C. R Ent. Belg 35 97 (1891)
*Kashmir

137 *Leptomorpha rugulipennis* Fairm. C. R Ent. Belg 35 97 (1891)
*Kashmir

138 *Lyprops indicus* Wiedem. in Bates Second Yarkand Mission, p 76 (1891)
*Jhelum Valley

139 *Myatis quadraticollis* Bates Second Yarkand Mission Coleop p 74 (1891)
*Between Leh and Yarkand.

140. *Ocnera protensa* Fairm. C. R. Ent. Belg 35 94 (1891)
*Kashmir

141 *Opatrum ochthebioides* Fauvel in Bates Second Yarkand Mission Coleop p 75 (1891)
Dras, Kargil, Leh.

142. *Prosodes rufo-sulcata* Fairm. C. R Ent. Belg 35 95 (1891)
*Kashmir

143 *Prosodes trisulcata* Bates Second Yarkand Mission Coleop. p. 63 (1891)
*Dras Kargil Leh.

144 *Prosodes ciccus* Bates Second Yarkand Mission Coleop p. 63 (1891)
Sind Valley

145 *Pseudoblaps simulatrix* Fairm. C. R Ent. Belg 35 100 (1891)
Kashmir

146 *Solskya caperiaceus* Gridelli, Atti Mus Stor nat. Triest, 12 53 (1935)
*Kashmir

147 *Synchir apicalis* Gridelli, Zoological results of The 3rd Danish Expedition to Central Asia. Tenebrionidae from Afghanistan.
Videnskabelige Meddelelser fra Dansk Nat. Forening 117 26 (1955)
High Valleys of Kashmir

148 *Syschus cuglae* Gridelli, Videnskabelige Meddelelser fra Dansk Nat. Forening 117 26 (1955)
*High Valleys of Kashmir

149 *Syschus himalaicus* Bates in Gridelli Videnskabelige Meddelelser fra Dansk Nat. Forening 117 26 (1955)
*High Valleys of Kashmir Dras Kargil Leh.

150 *Syschis piceicornis* Bates in Gridelli, Videnskabelige Meddelelser fra Dansk Nat Forening 117 26 (1955)
*Dras, Kargil Leh High Valleys of Kashmir

151 *Tetramillus simplicifrons* Gridelli, Atti Mus. Stor nat. Trieste, 12 33 (1935)
*Kashmir

FAMILY SCARABAEIDAE

152 *Copris sinicus* Hope, II Yarkand Miss Coleoptera (1891) Arrow FBI Lamellicornia 3 115 (1931)
*Murree C. P Sarda Rangoon Maymo North Chin Hills Pahu, Pegu Tenasserim Java Siam Tonkin S & E China.

153 *Gymnopleurus cyaneus* (Fab) Ent Syst. Suppl. p. 34 (1798) (*Copris*) II Yarkand Miss Coleoptera (1891) Arrow FBI Lamellicornia, 3 49 (1931)
*Jhelum Valley U P West Almora, Haldwani Chakrata, Arki 4000 ft Bengal Dacca, Calcutta Nagpur Belgaum Coimbatore, Bellary Malabar C Comorin Anamalai Hills Ceylon.

154 *Gymnopleurus mundus* Weid. in Sharp II Yarkand Miss Coleoptera, (1891) Arrow FBI Lamellicornia 3 57 (1931)
*Jhelum Valley Bihar Chapra South west China.

155 *Onthophagus armiceps* Reiche in Sharp II Yarkand Miss. Coleoptera (1891)
*Jhelum Valley

156 *Scarabaeus sylvaticus* Panz in Sharp II Yarkand Miss., Coleopetera (1891)
*Sind Valley

157 *Sisyphus hirtus* Wied Sharp II Yarkand Miss. Coleoptera (1891) Arrow FBI Lamellicornia 3 76 (1931)
*Jhelum Valley Bengal Berhampur Belgaum South India Sipur Palghat Bangalore, S. Mysore, Nilgiris Karkur Malabar Cole kandy Kitulgala Horawupotana Bitenne.

158. *Sisyphus neglectus* Gory Mon Sisyphus p 14 (1833) Arrow FBI Lamellicornia 3 73 (1931)
*Murree 5500 ft Bombay N Kanara Kathiawar Dharwar C P Chikalda UP Mussourie S Mysore

159 *Trox procerus* Har in Sharp II Yarkand Miss. Coleoptera (1891).
*Jhelum valley

FAMILY RUTELIDAE

160 *Adoretus limbatus* Blanch. in II Yarkand Miss. Coleoptera (1891) Arrow FBI Lamellicornia, 2 305 (1917)
*Jhelum Valley Calcutta Kathuar Dacca Berhampur Kanpur Burma Siam

161 *Adoretus pallens* Blanch II Yarkand Miss. Coleoptera (1891) (*audius rufus*) Arrow FBI Lamellicornia 2 334 (1917)
*Jhelum Valley Burma

162. *Anomala rufiventris* Redt. II Yarkand Miss. Coleoptera (1891) Arrow FBI., Lamellicornia, 2 236 (1917)
*Murree. West Almora Ranikhet, Kurseong Manipur Khasi Hills Jaintia Hills Darjeeling Mungphu Bhutan.

163 *Anomala stoliczkae* Sharp J R Asiatic Soc. Bombay 47 (?) 173 (1878) Arrow FBI Lamellicornia 2 245 (1917)
*Murree. UP Jaunsar Sikkim Darjeeling 7000 ft. Tibet West China.

FAMILY APHODIIDAE

164 *Aphodius costatus* Schmidt, Entomol Wochenbl 47 (1908) Kapur RIM 52 (2-4) 338 (1954)
*Kulu. Belgaum, Manipur

165. *Aphodius kashmirensis* Sharp II Yarkand Miss. Coleoptera, (1891)
*Dras Kargil, Leh.

166. *Aphodius parvulus* Har in Sharp II Yarkand Miss Coleoptera (1891)
*Jhelum Valley Ajmer Abyssinia

FAMILY GEOTRUPIDAE

167 *Geotrupes cremulipennis* Fairm. C R Ent Belg 35 122 (1891)
*Kashmir

168 *Geotrupes jakowlewi* Sem. in Schuster Hindukush Expedition Arb morp. taxon. Ent. Berlin-Dalhem 3 (3) 202 (1936)
Kashmir Chitral, Caucasus Turkestan, Bukhara

169 *Geotrupes Kashmirensis* Sharp II Yarkand Miss. Coleoptera, (1891)
*Dras, Kargil, Leh.

170 *Geotrupes orientalis* Hope, in Sharp II Yarkand Miss. Coleoptera (1891)
Murree.

171 *Geotrupes semicribrosus* Fairm. C. R. Ent. Belg 35 122 (1891)
*Kashmir

FAMILY MELOLONTHIDAE

172 *Phyllophaga (Lachnosterna) stoliczkae* Sharp II Yarkand Miss Coleoptera (1891)
Murree.

173 *Phyllophaga (Lachnosterna) stridulans* Sharp II Yarkand Miss., Coleoptera (1891)
*Murree.

FAMILY CERAMBYCIDAE

174 *Caloclytus ignobilis* (Bates) Proc. Zool. Soc. p 721 (1878) (Clytanthus) Gahan FBI Cerambycidae, 1 270 (1906)
*Murree

175 *Hesperophanes cribricollis*, Bates, Proc. Zool. Soc. p 720 (1878) Gahan, FBI Cerambycidae 1 113 (1906)
*Murree.

176 *Purpuricenus indus* Sem Rev Russe Ent. 7 139 (1888) Ahmed, T Indian J Ent 8 (1), 44 (1946)
*Kashmir Darra i-Khail Afghanistan.

FAMILY LUCANIDAE

177 *Cerackus alavus* Fairm. C R. Ent. Belg 35 88 (1891)
*Kashmir

178 *Eurytrachelus submolaris* Hope in Gravely RIM., 11 424 (1915)
*Murree. Naini Tal.

179 *Hemisodorcus suturalis* Westwood in Gravely RIM., 11 422 (1915).
*Kashmir Valley 5-6000 ft Dehra Dun Tehri Garhwal

FAMILY CHRYSOMELIDAE

180 *Byrkia flaviventre* (Baly) in Bates II Yarkand Miss., Coleoptera (1891) (*Malacosoma*) Maulik, FBI Chrysomelidae Galurecinae, p. 117 (1936)
*Murree

181 *Charoea flaviventris* Baly in Bates, II Yarkand Miss. Coleoptera (1891) Maulik, FBI Chrysomelidae Galurecinae, p 301 (1936)
*Murree.

182 *Galruca indica* Baly in Bates II Yarkand Miss. Coleoptera (1891) Maulik FBI Chrysomelidae Galurecinae p 101 (1936)
*Murree. North India Simla Hills, Mussourie Hazara Distt., Dunga Gali 8000 ft West Almora Assam

183 *Haltica caerulescens* (Baly) Trans. Ent. Soc London 190 (1874) (Graptodera) Maulik FBI Chrysomelidae Halticinae p 471 (1926).
Murree. Japan China.

184 *Haltica cyanea* Weber Obs. Entom 1 57 (1801) Maulik, FBI, Chrysomelidae Halticinae p 422 (1926)
*Chamba. Bombay Belgaum Burma Shwegoo, Katha Seetang Tenasserim, Thagata Jrugion Bhamo Ruby Mines Karen Mts. Sumatra.

185 *Haltica viridicyanae* Baly Trans Ent Soc. London 191 (1874) Maulik, FBI Chrysomelidae Halticinae, p 422 (1926)
Sind Valley Japan Nagasaki

186. *Nodostoma concinnicolle* Baly Cistula Ent 2 372 (1880) FBI Chrysomelidae 2 312 (1908)
*Jhelum Valley Kashgar Bengal, Mandar

187 *Nodostoma plagiatum* Baly Cist. Ent. 2 373 (1880) FBI Chrysomelidae, 2 315 (1908)
*Murree. Kashgar Assam Khasi Hills.

188. *Plagiodera versicolora* (Laich.) in Bates, II Yarkand Miss. Coleoptera (1891) Maulik, FBI Chrysomelidae p 61 (1926)
Jhelum Valley Europe Siberia, Africa, West Almora Abbotabad.

189 *Philopona signata* Duvivier Ann. Soc. Ent. Belg 36 429 (1892) (*Hyphasis*) Maulik, FBI Chrysomelidae Halticinae p 155 (1926)
*Kangra Valley Chota Nagpur Koubis, Bombay C P Sindevai Chandra Distt. Bengal Rajmahal.

190. *Phyllodecta abdominalis* (Baly) Cyst. Ent. 2 375 (1878) (*Phratora*) Maulik, FBI. Chrysomelidae Halticinae p 83 (1926)
*Murree 7500 ft UP Nainital Garhwal 6500 ft Sundardhunga Valley 8-12000 ft.

Order LEPIDOPTERA

Family Alucitidae

1 *Platyptilia superscandens* Fletcher Indian J Ent. 2(1) 11 (1940)
Kashmir Killanmarg 11000 ft.

Family Lycaenidae

2 *Lycaena phlaeas stygianus* Butler Proc. Zoo Soc London p 408 (1880) (*Chrysophanus stygianus*) Ahmed Taskhir Indian J Ent. 8(2) 208 (1946)
Kashmir Nainital Kumaon, Mussourie Simla Quetta, Afghanistan N W Europe.

Family Hesperiidae

3 *Carcharodus alceae swinhoei* Watson, Proc. Zool Soc. London p 68 (1893) Ahmed Taskhir Indian J Ent. 8(2) 209 (1946)
*Kashmir Chitral Baluchistan Afghanistan.

Family Noctuidae

4 *Apopestes spectrum* (Esp.) Die Europaische Schmetterlinge, 4(1) 131 pl. 100 figs 3 4 Ahmed Taskhir Indian J Ent., 8(2) 210 (1946)
*Murree, Dharmsala. Quetta, Mainpuri Europe Syria Asia Minor Armenia Turkestan

5 *Catocalla afghana* Swinh., Trans. Ent. Soc. London p. 352 (1885) Ahmed, Taskhir Indian J Ent. 8(2) 211 (1946)
Kashmir Rajaori. Baluchistan Afghanistan Kabul Kandhar

Order HYMENOPTERA

Family Tenthredinidae

1 *Allantus multicolor* Smith Second Yarkand Mission Hymenop. (1878)
*Murree.

2 *Allantus providus* Smith II Yarkand Mission Hymenop. (1878)
*Murree.

3 *Allantus simillimus* Smith II Yarkand Mission Hymen. (1878.)
*Murree

4 *Allantus terminalis* Smith II Yarkand Mission Hymen., 1878.
*Kashmir Sind Valley

5 *Hylotoma fumipennis* Smith II Yarkand Mission Hymen., 1878.
*Jhelum Valley

6 *Macrophya opposita* Smith II Yarkand Mission, Hymen 1878.
*Kashmir Sind Valley

7 *Tenthredo fallax* Smith, II Yarkand Mission Hymen., 1878.
*Sind Valley Murree.

8. *Tenthredo nigro-maculatus* Smith II Yarkand Mission, Hymen, 1878.
*Kashmir Sind Valley

9 *Tenthredo simulata* Smith II Yarkand Mission Hymen., 1878.
*Kashmir Sind Valley

Family Ichneumonidae

10 *Cryptus insidiator* Smith II Yarkand Mission Hymen., 1878.
*Kashmir Sind Valley

11 *Ichneumon bimaculatus* Smith II Yarkand Mission Hymenop. 1878.
*Murree

12. *Paniscus renovatus* Smith II Yarkand Mission Hymenoptera, 1878 (*ru-color*) Morley FBI 3 (1) 354 (1913)
*Kashmir Sind Valley Murree.

SUPERFAMILY SPHECOIDEA

Family Sphegidae

13 *Ammophila vagabunda* Smith Cat, iv :218 II Yarkand Mission, Hymen. 187 *spinipes*) FBI Hymenoptera 1:231 (1897)
*Dras, Kargil Leh North China Sumatra.

SUPERFAMILY APOIDEA

Family Pompilidae

14 *Pompilus atripes* Smith II Yarkand Mission Hymenop 1878 FBI H men-optera, 1 163 (1897)
Murree

15. *Proceramus rufo-femoratus* Smith, II Yarkand Mission Hymenop 1878
Dras Kargil Leh

Family Bombidae

16 *Bombus alticus* Eversm. in Smith, II Yarkand Mission Hymenop 1878
*Tankise Pangong Valley Ladakh Asiatic Russia

SUPERFAMILY FORMICOIDEA

Family Formicidae

1 *Acantholepis frauenfeldi integra* Forel J Bombay Nat. Hist. Soc 8 411-415 (1894) Bingham FBI Hym 2.316 (1903) Eidmann Zool Jahrb 75 249 (1942)
*Talichi near Nanga Parbat, 4290 ft (1300 m)

2. *Acantholepis frauenfeldi var seric a* Fr in Bingham FBI Hym. 2 316 (1903) Menozzi Atti, Soc. Ital Mus Civ Milano 78 339 (1939)
Kashmir Jhelum Valley Takht i-Sulaiman 7260 ft. (2200 m.) Dusso 7970 ft. (2400 m.)

3 *Bothriomyrmex myops* For in Menozzi Atti Soc Ital. Mus. Civ Milano 78.338 (1939)
*Kashmir Sikkim Hindostan.

4 *Camponotus aethiops var cashmiriensis* For n Menozzi, Atti, Soc. Ital. Mus Civ Milano 78 379 (1939)
*Kashmir

5 *Camponotus (Camponotus) japonicus aterrima* Emery Ann. Mus Stor Genova, 34 478 (1894) Eidmann Zool Jarhb 75 250 (1942)
*Turbaling near Nanga Parbat 8910 ft (2700 m) Tibet, Manchuria, China

6 *Camponotus mitis var bacchus* (Smith) in Second Yarkand Mission, Hymenoptera 1878 FBI Hym. 2.356 (1903)
Jhelam Valley Calcutta Ceylon, Islands of Eastern Archepalongo.

7 *Camponotus sericeus* For n Menozzi Atti Soc Ital Mus Civ Milano 78.341 (1939)
Kashmir Srinagar 6600 ft (2000 m)

8 *Camponotus sylvaticus paradichroa* Em. n Menozzi Atti Soc Ital. Mus Civ Milano 78.315 (1939)
Jhelam Valley 3960 ft. (1200 m) Sooroo 8910 ft. (2700 m) Karakoram Braldo Valley Askole, 10230-10560 ft. (3100-3200 m)

9 *Cataglyphus (Cataglyphus) bicolor setipes* Forel J Bombay Nat Hist. 8 401 (1894) Bingham FBI Hym. 2 312 (1903) Eidmann, Zool. Jahrb 75:254 (1942)
Gor near Nanga Parbat, 7920 ft. (2400 m) North Africa S Europe, N W F P

10 *Cataglyphus (Monocombus) cugiai* Menozzi Atti. Soc Ital Mus Civ Milano 78.323 (1939)

*Dras 10230 ft. (3100 m.) Kargil 8910 ft. (2700 m) Tartalgarh near Nanga Parbat 9240 ft (2800 m) Skardu 7260 ft. (2200 m) Braldo Valley Dussu 7920 ft. (2400 m) Askole 10395 ft. (3150 m) Pummah Valley Skiniltalmosa 10560 ft. (3200 m)

11 *Cataglyphis hispidus* Menozzi in Eidmann, Zool. Jahrb 75:255 (1942).
*Gor near Nanga Parbat 8910 ft. (2700 m)

12 *Crematogaster apicalis* Smith, II Yarkand Mission. Hymenoptera p.12, 1878; Bingham FBI 2 147 (1903)
*Jhelam Valley

13 *Crematogaster subdentata kashgariensis* Forel in Menozzi Atti. Soc. Ital. Mus. Civ Milano 78.335 (1939)
*Kashmir Jhelam Valley 3960 ft. E. Turkestan.

14 *Dorylus labiatus* Shuck in Smith, II Yarkand Mission. Hym., 1878 (larvae)
Bingham, FBI 2.2 (1903)
*Jhelam Valley Whole of Continental India.

15 *Formica fusca ssp glebaria var rubescens* For in Menozzi, Atti. Soc. Ital. Mus. Civ Milano 78.343 (1939)
*Kashmir Srinagar 5610 ft. (1700 m.) Baltal 9570 ft. (2900 m) Lalpani (Deosal) 13200 ft (4000 m) Dras 10230 ft. (3100 m) Chamara near Nanga Parbat 10560 ft. (3200 m) Kangra 5170 ft. (1900 m) Gongma 8745 ft. (2650 m) Shirting 8250 ft. (2500 m) Hot Sulphur Spring Chongo 9900 ft. (3000 m) Shigar 7260 ft. (2200 m)

16 *Formica (serviformica) picea* Nyl. in Menozzi, Atti Soc. Ital. Mus. Civ Milano, 78.320 (1939)
*Sind Valley Kishanganga Valley Chota Deosal 12705 ft. (3850 m) Lalpani 13200 ft. (4000 m) Kamri 7920 ft. (2400 m) Burzil Chauki 11220 ft. (3400 m) Braldo Valley Hoto 9240 ft. (2800 m) Hot Sulphur Spring Chongo 9900 ft (3000 m), Askole 10230 ft. (3100 m) Thla Brok 13200 ft. (4000 m) Pummah Valley Tsok 9240 ft. (2800 m) Dumultar 12870 ft. (3900 m) Skinmag 14190 ft. (4300 m) Baltoro Confluence of Baltore Dunge 12870 ft (3900 m) Liligo 12540 ft. (3800 m) Robutze 12210 ft (3700 m) Urdukas 13200 ft (4000 m) Munda 14190 ft (4300 m) Jermanendu 14190 ft (4300 m) Mustag 15840 ft. (4800 m) Moni Bransa 15180 ft. (4600 m) Durbin Jangal 13200 ft. (4000 m) Valley of Mt. K2 13860 ft. (4200 m.) Indus Valley Karal Mark 1419 ft. (4300 m) Boorgi Nullah 11880 ft. (3600 m)

17 *Formica truncorum* Fab Syst. Piez p 403 (1804) Eidmann, Zool. Jahrb 75.253 (1942)
*Gor near Nanga Parbat 9240 ft (2800 m) Himalaya. Central Europe Sino China

18 *Lasius bicornis kashmirensis* Donish. in Menozzi Atti. Soc. Ital. Mus. Civ Milano 78 342 (1939)
*Kashmir

19 *Leptothorax wroughtoni* Forel in Menozzi Atti. Soc Ital. Mus. Civ Milano 78.337 (1939)

*Liddar Valley

20 *Monomorium insae* Forel, in Menozzi Atti. Soc. Ital. Mus. Civ. Milano 78 336 (1939)
*Kashmir

21 *Monomorium (Parholcomyrmex) destructor* Jerd. Madras J. Litter Soc. 17 105 (1851) Bingham FBI 2.209 (1903) Eidmann Zool. Jarhb 75 248 (1942)
*Drang near Nanga Parbat 3960 ft. (1200 m) Throughout Indian limits

22 *Mutilla suspiciosa* Smith, II Yarkand Mission Hymenoptera 1878
*Jhelam Valley Borneo, Celebes Batchian Amboyna, Bouru

23 *Myrmica rugosus-sabandiae* Menozzi, Atti Soc. Ital. Mus Civ Milano 78:286 (1939) Eidmann, Zool Jarhb 75 245 (1942)
*Kashmir Gund (Sind Valley) 6864 ft. (2080 m) Doyan near Nanga Parbat 8910 ft. (2700 m) Askole 10230 ft. (3100 m) Tolti 7920 ft. (2400 m) Skardu 7260 ft. (2200 m) Kutty Kushumul 7590 ft. (2300 m) Dumo 7250 ft (2200 m)

24 *Myrmica luctuosa* Smith, II Yarkand Mission, Hymenoptera, 1878 Bingham FBI. 2:212 (1903)
*Murree.

25. *Myrmica smythiesi cachmirensis* For in Menozzi Atti. Soc. Ital. Mus Civ Milano 78.331 (1939)
*Kashmir Chokpiong 8580 ft. (2600 m) Askole 10230 ft. (3100 m) Snimtsa 10560 ft. (3200 m) Trok (Purmah Valley) 11550 ft. (3500 m)

26 *Myrmica smythiesi fortior* For in Menozzi Atti. Soc. Ital. Mus Civ Milano, 78.331 (1939)
Kashmir Eastern Siberia

27 *Myrmica smythiesi lutescens* Forel in Menozzi Atti. Soc. Ital. Mus. Civ Milano 78.331 (1939)
Kashmir Kangan 6600 ft. (2000 m) Trok 11550 ft. (3500 m)

28. *Pheidole jucunda dharmsalana* For in Menozzi Atti. Soc. Ital. Mus. Civ Milano 78.333 (1939)
N W Himalaya.

29 *Pheidole (Pheidole) Robertsi* Forel Rev Suisse, Zool 10 183 (1902) Bingham, FBI Hym. 2:259 (1903)
*Bunji near Nanga Parbat, 4620 ft. (1400 m) Kanara Sikkim.

30 *Plagiolepis balestrieri* Menozzi, Atti Soc Ital. Mus. Civ Milano 78 310 (1939)
*Kashmir Kargil 89100 ft. (2700 m) Skardu 7260 ft. (2200 m) Karakorum Shigar Khatty 7590 ft. (2300 m) Hot Sulphur Spring Chongo, 9900 ft. (3000 m) Askole 10230 ft. (3100 m) Kro Brok 12210 ft.(3700 m)

31 *Tapinoma wroughtoni* Forel, in Menozzi Atti Soc. Ital Mus Civ Milano 78.338 (1939)
Kashmir Margundo (Sind Valley) 6600 ft. (2000 m) Gor near Nanga Parbat 8910 ft. (2700 m)

32 *Tetramorium caespitum himalayana* Wiehm. Arch, f. Naturgesch, A, 79 (11) 58 (1913) Eidamann Zool Jarhb 75:248 (1942) Mushkin near Nanga Parbat 7590 ft. (2300 m) Himalaya.

33 *Tetramorium elisbethae* Forel in Menozzi, Atti Soc. Ital. Mus. Civ Milano, 78.337 (1930)
*Kashmir

Order DIPTERA

Family Calliphoridae

1 *Calliphora aucta* Walker in Senior White, RIM., 28 129 (1926)
*Kashmir above 5000 ft. Kashmir to Khasi Hills, throughout Himalaya

Family Bibionidae

2 *Dilophus graciosus* Big J Asiatic Soc. Bengal 59:265 (1890) FBI p. 178, Brunetti RIM 17 41 (1920)
*Dharmsala. Kurseong Phagu Theog Bhowali Kumaon Upper Burma Central India Yunnann S China.

Family Blepharoceridae

3 *Philorus bionis* Agharkar RIM 10 160 (1914)
*Kashmir Nagaheran

Family Ephydridae

4 *Ephydra glauca* Meigen, Syst Beschr Europ Zweifl 6 120 (1830) Cresson, Mem. Conn Acad. Arts & Sci 10 1 (1934)
*Indian Tibet Tso-Kar 14850 ft. Europe Rumania, Central Asia, South Russia

5 *Ephydra tibetensis* Cresson Mem. Conn Acad. Arts & Sci. 10:2 (1934)
*Indian Tibet Kyam Hot Spring 15650 ft. Phuga Hot spring, 14500 ft.

6 *Holmopota hutchinsoni* Cresson Mem Conn. Acad. Arts & Sci., 10:3 (1934)
*Indian Tibet Tso-Kar 14800 ft

Following is the list of the species listed by Kollar and Redtenbacher in Hugel's Kaschmir which have not been included in the check-list.

Buprestidae

1 *Sternocera dasypleuros* Caschmir
2 *Lampetis coerulea*
3 *Agrilus caschmirensis*

Elateridae

4 *Lacon brachychaetus*
5 *Ludius caschmirensis*
6 *Cardiophorus vicinus*
7 *Cardiophorus consentaneus*
8 *Cardiophorus stolatus*

Lampyridae

9 *Lycus suturalis* Caschmir
10 *Celaphotis italica*

Cantharidae (Telephoridae)

11 *Cantharis caeruleo-maculata* ,,
12. *Aulostelus bimaculatus* Hope
13. *Deromma melanura*

Silphidae (Silphales)

14 *Silpha ioptera* ,,

Stenelytra

15 *Strongylium rufipenne*

Mylabridae (Trachelides)

16. *Lagria aenea*
17 *Lagria variabilis*
18. *Lagria bicolor*
19 *Epicauta rubriceps* ,,
20. *Epicauta limbata*
21 *Prionotus proracutus* ,,
22 *Prionotus serratulatus* ,,

Xylophagi

23 *Trogosita orientalis*

Aphidiphagi

24 *Coccinella borealis*
25 *Epilachna ocellata*
26 *Epilachna decemaculata*

THE ENDOSKELETON OF CATLA CATLA (HAM.)

PART I—THE SKULL

VEER BALA RASTOGI
Zoology Department, Meerut College, Meerut

INTRODUCTION

On the cyprinoid fishes complete osteology of *Labeo rohita* alone is available and need for accounts of a few more common forms is being urgently felt. The present work on the endoskeleton of common carp *Catla catla* (Ham.) is an attempt in that direction.

The fish is widely distributed, being represented in the rivers, streams and reservoirs throughout India. It is used profitably in pisciculture, owing to its nonpredatory habits and to its being the quickest growing freshwater fish in India. The convenient size and easy procurability renders it a suitable type for class study.

The investigations were carried out in the Zoology Department of Meerut College under the direction of Dr B. M. Sinha. It is my pleasant duty to thank him for the guidance in the work and to the authorities of the College for providing the necessary facilities.

THE SKULL

The skull is well ossified and hemispherical with eversible jaws. Its upper surface is rugose.

The Occipital Region

The occipital region consists of the usual four replacing bones, the supraoccipital above, basioccipital below and exoccipitals on sides.

The *supraoccipital* (I, II, III, IV & VI 1) is a flattened bone produced behind into a pair of laterally compressed processes. It bears the backwardly directed occipital spine, which approaches the neural spine of complex vertebra. On either side of the occipital spine the bone is provided with a faint groove. The supraoccipital articulates with the parietals, epiotics and exoccipitals.

The *basioccipital* (II, III, IV & VI 2) is a large bone distinguished into the anterior, middle and posterior parts. The middle part bears the occipital condyle, the deeply concave posterior surface of which articulates with the complex vertebra. The posterior part takes the form of a drain, which lodges the dorsal aorta in its course backwards. On its ventral surface,

this part bears an oval plate like masticatory process for the attachment of horny pad. The anterior part is more or less cylindrical and it carries a pair of depressions in front and a pair of channels behind. The depressions separated by a median ridge form parts of the foveae sacculi while the channels constitute the atria sinus imparis. The median ridge widens in its anterior part and depresses to form the floor of the cavum sinus imparis. The basioccipital articulates with the parasphenoid, exoccipitals and complex vertebra

The *exoccipital* (I II III IV & VI 9) is distinguished into the basal part and the laterally directed paroccipital process. The basal part bears an arched process, which encloses an oval fenestra covered by thick tough membrane. A process arises from the inner side of the basal part and meets the similar process of the other bone to enclose between them the cavum sinus imparis Below the basal part is a depression which together with a similar depression on basioccipital forms the fovea sacculi. The paroccipital process is flat and wing like and it articulates at its distal end with the pterotic process. An elongated foramen lies beneath the exoccipital for the ninth and tenth cranial nerves and behind this is the jugular foramen. The bone also bounds the subtemporal fossa mesially The two exoccipitals meet in the middle line excluding the supraoccipital from bounding the foramen magnum. The bone articulates with the supraoccipital, epiotic and basioccipital

The *Auditory Region*

The auditory region is ossified by four replacing bones, the prootic, epiotic sphenotic and pterotic. The opisthotic is absent.

The *prootic* (III IV & VI-4) is a large flattened bone on the ventral side of the auditory region forming its antero-mesial boundary The bone may be roughly distinguished into a thinner outer and thickened inner part. The latter bears a tunnel for the anterior semicircular canal of internal ear The bone is perf rated in front for the trigeminofacial complex of the fifth and seventh cranial nerves. The prootic articulates with the pleurosphenoid, parasphenoid sphenotic pterotic, epiotic and exoccipital.

The *epiotic* (I II III IV & VI-6) lies in the posterior part of the auditory region On its ventral surface the bone has a bowl-shaped cavity for accommodation of the posterior semicircular canal From the dorsal surface of the bone arises a small process which articulates with the posttemporal. The epiotic articulates with the parietal pterotic exoccipital and basioccipital.

The *sphenotic* (III IV & VI-6) lies in the anterior part of auditory region forming the posterior boundary of orbit It can be distinguished into the main part and the forwardly directed process The main part bears a groove which together with a similar groove in the pterotic, forms a depression for artic-

lation of the head of hyomandibula A small cavity towards the inner side of the bone forms a part of the auditory recess The main part of the bone articulates with parietal prootic and pleurosphenoid, while the process running along with the frontal is applied to it.

The *pterotic* (I II III & VI 3) is a large bone in the postero-lateral part of auditory region The bone can be distinguished into the anterior main part and the posterior pterotic process The main part lies on the outer side of the parietal and bears a facet for the hyomandibula. A prominent depression on the inner side forms a part of the subtemporal fossa and accommodates the horizontal semicircular canal of the internal ear The pterotic process is applied to the paroccipital process of exoccipital.

The *Sphenoidal Region*

The sphenoidal region comprises of the paired parietals frontals pleurosphenoids and orbitosphenoids and the unpaired parasphenoid. Of these the pleurosphenoids and orbitosphenoids are replacing bones and the rest are investing ones.

The *parietal* (I, II, IV & VI 7) is a large rectangular bone, which roofs over the cranium. Its dorsal surface is rugose, while the posterior is grooved. The bone articulates with the frontal, pterotic epiotic and supraoccipital

The *frontal* (I II III IV & VI-8) is a large flat bone which roofs the cranium in front of parietal. Its dorsal surface is rugose; while from its ventral surface arise articular surfaces for the pleurosphenoid and sphenotic Its anterior margin is convex and fits in the concave posterior surface of ethmoid. The frontal articulates with the ethmoid, lateral ethmoid, pterotic and parietal.

The *pleurosphenoid* (IV & VI 12) is distinguished into a horizontal basal piece and a vertical process. The basal pieces from two pleurosphenoids meet in the middle line leaving a notch for the posterior process of orbitosphenoid The vertical process of the bone getting attached to the ridge of frontal forms the side wall of the orbit. The bone also contributes to the formation of the groove for the head of hyomadibula. The pleurosphenoid articulates with the orbitosphenoid sphenotic, prootic and frontal.

The *orbitosphenoid* (III IV & VI 11) is a compound bone formed of two lateral elements. It lies in front of the pleurosphenoids and contributes to the floor and inner wall of the orbits. The bone is in the form of a horizontal plate with two lateral ridges raised upwards and fused to the frontals. It bears a groove on the ventral surface into which is received the ridge on the stem of parasphenoid. The orbitosphenoid articulates with the lateral ethmoids and pleurosphenoids.

The *parasphenoid* (III, IV & VI 13) is an elongated bone extending from the basioccipital to the vomer It consists of the body and the forwardly directed stem. The body is elongated and rhomboidal in form with a median ridge above and a better developed similar ridge below These ridges extend on to the stem of the bone. The body articulates with the exoccipitals behind and prootics and pleurosphenoids on the sides while the stem is applied to the orbitosphenoid above and the vomer in front.

The posterior *myodome* for the eye muscles is present, which is formed by the parasphenoid on the inner side, by the pleurosphenoid on the anterior and outer side and by the prootic on the posterior and inner side.

The *Orbital R gion*

The orbit is large and is bounded dorsally by the frontal, anteriorly by the lateral ethmoid, posteriorly by the sphenotic, and on the inner side by the orbitosphenoid and pleurosphenoid. In relation with the orbit are developed six orbital bones. These are the supraorbital, lacrymal and four suborbitals

The *supraorbital* (I II III & VI 15) is a flat bone lying attached to the side of the frontal It articulates with the lateral ethmoid and the third and fourth suborbitals.

The *suborbitals* (I II III & VI 18 19 20 21) are four splint-like curved bones forming the anterior outer and posterior boundary of the orbit Through them passes the infra orbital trunk of the lateral line system in its cou se from the lacrymal to pterotic. The last suborbital is applied by its whole length to the side of the frontal.

The *lacrymal* (I II III & VI 14) is a small scale-like bone more or less oval in form and with a slightly convex dorsal surfac It articulates with the maxilla, lateral ethmoid and first suborbital

The *Ethmoidal Region*

Th ethmoidal region is ossified by the paired nasals, lateral ethmoids and preethmoids and the median vomer rostral and ethmoid. The lateral ethmoids and preethmoids are replacing bones, while the rest are investing ones.

The *ethmoid* (I II III & VI 16) is a median transversely elongated bone the roof of the ethmoidal region It consists of a central body and two lateral flat wings. Anteriorly the bone presents a median notch bounded by a pair of ethmoid cornua Posteriorly a deep cleft separates the bone into the dorsal plate applied to the frontal and the ventral plate extending to the vomer The cleft is divided into a median and two lateral cavities. The median cavity forms the anterior part of the cranial cavity while the lateral cavities ...

the front ends of the olfactory capsules. The bone articulates with the nasals, frontals, lateral ethmoids, vomers and palatines.

The *lateral ethmoid* (II III IV & VI 17) lies on the side of the ethmoid and frontal. Each is distinguished into a main part excavated by a deep nasal pit and an outwardly directed Y-shaped process. The two bones articulate mesially below the parasphenoid and form the anterior boundary of the orbit. The lateral ethmoid articulates with the orbitosphenoid lacrymal and supra orbital.

The *nasal* (I II & VI 22) is a small, scale-like bone lying on the inner side of the olfactory capsule in the notch formed by the ethmoid and frontal.

The *rostral* (I II III & VI 24) is a small, rod-shaped bone lying in front of the ethmoid. Its anterior end lies between the rostral processes of maxilla and the posterior end fits in the notch of ethmoid.

The *vomer* (III IV & VI 10) is a thin flat and more or less triangular bone, lying on the ventral surface of the skull in front of the parasphenoid. Its anterior border is slightly concave bearing a thick condyle on either side for muscle attachment. On the outer side of the condyle is a small preethmoid bone. The vomer articulates with the parasphenoid palatine and lateral ethmoid.

The Visceral Skeleton

The visceral skeleton consists of the mandibular hyoid and five bran chial arches. The first four branchial arches are of the usual type while the fifth is gill-less and is represented by the inferior pharyngeal bones only

The *Mandibular Arch*

The mandibular arch is ossified by the primary endoskeletal and secondary dermal bones. The dorsal *palatopterygoquadrate* part, which forms the upper jaw is replaced by the palatine metapterygoid and quadrate and invested by the premaxilla, maxilla ectopterygoid and endopterygoid. The ventral *Meckel'* cartilage forms the lower jaw and is ossified by the dentary angular sesamoid angular and retroarticular

The *palatine* (III V & VII 1) is narrow in the middle and expanded at the two ends. On its inner side is a small projection to articulate with the vomer Its anterior end bears articular surfaces for the preethmoid and maxilla, and the posterior end for the entopterygoid and ectopterygoid.

The *ectopterygoid* (V & VII 2) is a small, triangular bone which has an articular notch on its anterior face for the palatine and a surface along its inner side for the entopterygoid.

The *metapterygoid* (I V & VII 3) is a large, plate-like bone on the outer side of the entopterygoid and ectopterygoid and above the hyomandibula. Below it articulates with the symplectic.

The *entopterygoid* (I, V & VII-4) is a thin, membrane-like bone, which bears along the anterior face a thickened surface for the ectopterygoid. To the upper side of the thickened surface is applied the palatine Externally its lower surface is partly overlapped by the metapterygoid.

The *quadrate* (I V & VII 11) is a somewhat triangular bone with a double condyle at the anterior end, to articulate with the angular On the inner side of the bone runs a ridge obliquely backwards from the condyle and below this ridge is a narrow groove into which fits the process of symplectic. The bone articulates with the angular ectopterygoid, symplectic and preoperculum

The *premaxilla* (I, II III & VII-8) is a thin curved flattened bone meeting with the fellow of the opposite side medially It may be distinguished into a horizontal limb directed towards the rostral and a lateral limb lying below the maxilla The posterior margin of the bone is deeply concave The distal end of the lateral limb together with the lateral limb of maxilla is attached to the dentary by means of ligaments

The *maxilla* (I II III & VI 7) is a transversely elongated bone, lying parallel to premaxilla, partly above and behind it. It shows as many as five processes two infront, two behind and one on the inner side. The two anterior processes articulate with the premaxilla and rostral bones. Of the two posterior processes the outer process articulates with the preethmoid and vomer and the inner process with the lacrymal and lateral ethmoid. The fifth process articulates with the dentary The two maxillae are joined *in the middle line* by the connective tissue and between them lies the rostral bone.

The *dentary* (I V & VII 5) is a broad bone, curved in the form of a cup and it bears the coronoid process. In its concavity is a groove for the angular The two dentaries meet medially into the mandibular symphysis.

The *angular* (I V & VII-6) is a thin flat bone with thickened proximal part. The bone is overlapped by the dentary except for the proximal part, which bears an articular facet for the quadrate. The inner surface of the bone bears a groove in which lies the *sesamoid angular* Along the proximal part of the bone is a triangular *retroarticular* bone. The mandibular canal of the lateral line system passes from this bone and ends at anterior end of dentary

The *Hyoid Arch*

Each half of the hyoid arch is divisible into dorsal hyomandibular part and the ventral hyoid cornu. The former ossifies into the hyomandibular and

symplectic, while the latter consists of the epihyal ceratohyal and hypophyals.

The *hyomandibula* (I V & VII 9) is an elongated prominent bony rod which lies obliquely from the auditory region suspending the two jaws. The proximal end of the bone is produced into an elongated head bearing two closely set condyles which articulate with the facet in the sphenotic and pterotic. On its posterior face is a knob-like condylar head for the operculum The posterior surface of the bone is partly covered by the preoperculum and operculum. The bone articulates in front with the symplectic

The *symplectic* (I V & VII 10) is a long narrow bone below the quadrate and metapterygoid and in front of hyomandibula. Its anterior two-third fits into the groove of the quadrate and externally it is covered by the preoperculum.

The *epihyal* (VIII) is a flattened bone appended to the hyomandibula and symplectic by its outer end On its posterior surface it carries the first branchiostegal ray

The *ceratohyal* (VIII) is a flat bony piece lying in front of the epihyal articulating anteriorly with the two hyohyals. It bears along its posterior border the second and third branchiostegal rays.

The *hypohyals* (VIII) are two small bony nodules in front of the ceratohyal lying one over the other The ventral piece is slightly larger than the dorsal. The two ventral hypohyals are connected in the middle line, while the two dorsals carry the basihyal in between

The *basihyal* (VIII) is an elongated cylindrical rod extending forward from the dorsal hypohyals to support the tongue

The *branchiostegal-rays* (VIII) are three long sabre-shaped bones supporting the branchiostegal membrane. They are directed backwards and outwards and decrease in size and thickness from first of the series to the last

The *urohyal* (VIII) is a median roughly triangular bone lying on the floor of buccal cavity Its narrow anterior end is attached to the ventral pair of hypohyals, while the rest extends below the ceratobranchials of two sides From the upper surface of the bone arises a vertical ridge, which gradually increases towards the distal end The posterior end of urohyal is slightly bifurcated

In relation with the hyoid arch are developed four investing bones the operculum interoperculum, preoperculum and suboperculum, which constitute the gill cover

The *operculum* (I V & VII 15) is the largest bone of the series with dorsal convex and ventral concave surfaces The bone has an articular facet for

hyomandibula at its anterior border On the inner side of the bone are present a number of shallow depressions for the attachment muscles

The *preoperculum* (I, V & VII 14) is a large curved bone lying in front and above the operculum. It is slightly raised on its outer surface marking the position of operculo-mandibular sensory canal of the lateral line system. Its upper end is rounded and blunt and articulates with the hyomandibula, while the lower is slightly pointed and is overlapped by the quadrate and symplectic

The *suboperculum* (I, V & VIII 13) is an elongated sabre-shaped bone lying partly below and internal to the operculum. The anterior end of the bone is overlapped by the interoperculum.

The *interoperculum* (I V & VII 12) is a plate like bone lying along the lower border of the preoperculum partly overlapped by it.

The *Branchial Arches*

The *pharyngobranchials* (VIII) are rounded bony pieces lying obliquely on the dorsal wall of the pharynx. The pharyngobranchials of the first arch are well developed, of the second and third fused and of the fourth arch are reduced.

The *epibranchials* (VIII) are curved elongated rods lying obliquely on the roof of the phraynx directed backwards from the pharyngobranchials. They are grooved along their dorsal surface, for the branchial arteries and ventrally bear gill rakers in double rows. The proximal ends of the epibranchials are swollen for attachment to the skull along with the pharyngobranchials.

The *ceratobranchials* (VIII) are elongated, curved rods directed forwards and inwards on the floor of the pharynx. The ceratobranchials carry dorsally double rows of gill rakers and are grooved ventrally for the branchial arteries. The ceratobranchials of the first arch meet on the first basibranchial, of the second and third are attached to the second basibranchial and of the fourth arch meet in the middle line

The *hypobranchials* (VIII) are small bony nodules present in relation with the ceratohyals of first arch and attached to the dorsal pair of hypohyals.

The *basibranchials* (VIII) lie in the median cartilage between the elements of two sides There are only two of them one behind the other

The *inferior pharyngeal bones* (VIII) are more or less triangular bones having their ventral surfaces perforated On the dorsal side they bear teeth in three rows five in the first row four in the second and two in the third row. The inferior pharyngeal bones are suspended from the urohyal by means of cartilage.

Summary

The skull is completely edentulous, platybasic and sufficiently ossified. The cranial roof is uninterrupted and jaws are eversible. The ophthotic, basisphenoid and interhyal are absent, while the symplectic and posterior myodome are distinct.

The supraoccipital spine is not well developed. The exoccipital bears a jugular foramen and common foramen for the ninth and tenth cranial nerves. The two exoccipitals form the roof of the foramen magnum as well. The basioccipital is greatly modified in connection with the Weberian apparatus, bearing cavum sinus imparis, atria sinus imparis, sinus endolymphaticus and foveae sacculi. The sphenotic does not contribute to the cranial roof as the frontal meets the pterotic posteriad. The prootic, sphenotic, pterotic and pleurosphenoid contribute to the formation of the hyomandibular facet. The epiotic gives out a backwardly directed process, which articulates with the posttemporal. The orbitosphenoid is a compound bone formed by the two elements. In connection with the orbit are developed the four suborbitals, a lacrymal and a supraorbital. A preethmoid is present on either side of the vomer. The lateral ethmoid is hollowed out for the passage of the olfactory nerve and the rostral is small and rod like. The ethmoid is produced into lateral ethmoid cornu and a median notch for rostral. The vomer extends out anteriorly beyond the ethmoid and is visible on the dorsal view of the skull.

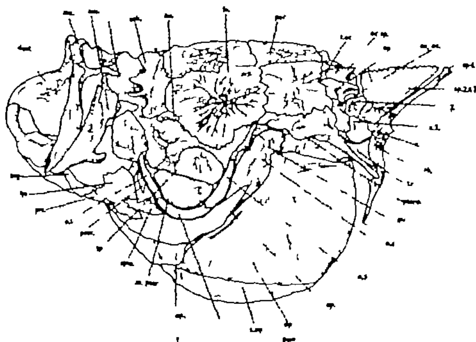
The lower jaw is ossified by the dentary, angular, retroarticular and sesamoidangular. The pterygoid is absent but the ectopterygoid, entopterygoid and metapterygoid bones are present. The hyomandibula gains articulation with the sphenotic-pterotic region by two facets. The premaxillae alone form the upper gape of the mouth. The opercular series is formed of usual four bones.

The basihyal is attached to the dorsal pair of hypohyals. There are three branchiostegal rays. The pharyngobranchials of first arch are not well ossified and of the 2nd and third arch are fused. The hypobranchials are ossified only in the first arch and there are only two basibranchials. The fifth branchial arch is represented by inferior pharyngeal bones bearing teeth.

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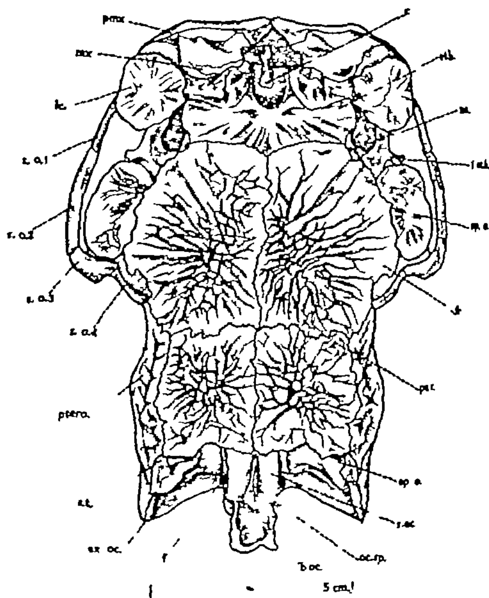
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Sid view f the skull

ang. ?ngular d., dentary; ep. epiotic; ent.pter. entopterygoid; eth. ethmoid; ex.oc. exoccipital; fr., frontal; h.m. hyomandibula; i.op. interoperculum; m.pter. metapterygoid; mx. maxilla; na. nasal; 2 3 4 eural ribes of second third and fourth vertebrae; n.sp. 2 & 3., neural spine of second and third vertebra; n.sp.4 neural spine of fourth vertebra; op. operculum; oc.sp. occipital spine; par. parietal; pmx., premaxilla; p.op. preoperculum; ptero. pterotic; pl.rib.4 pleural rib of fourth vertebra; qu., quadrate; rostral; art., retroarticular; s.oc. supraoccipital; 1 2 3 4., first, second third and fourth suborbitals; s.op. suboperculum; s.o. supraorbital; sym., symplectic; tr. tripus.

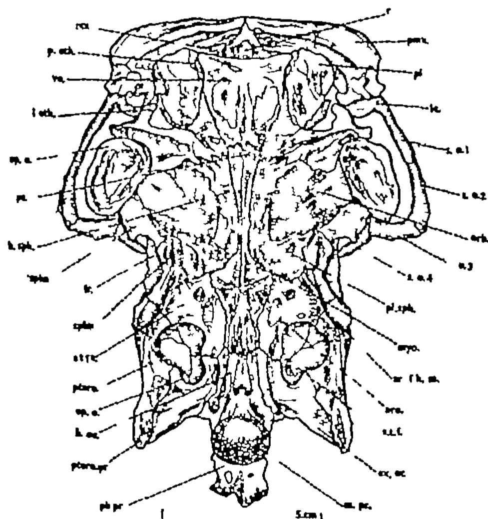
Catla catla (Ham.) Plate-II



Dorsal view of the cranium

b oc. basioccipital *ep o* epiotic *eth.* ethmoid; *ex. oc* exoccipital *f* foramen; *fr* frontal *lc.*, lacrymal *l. eth* lateral ethmoid *mx.* maxilla *na.*, nasal *oc. sp* occipital spine *par* parietal *ptero.*, pterotic; *pmx* premaxilla [illegible] rostral *s* [illegible] second, third and fourth suborbitals *s oc* supraoccipital *sp o.*, [illegible] supratemporal.

Catla catla (Ham.) Plate-III



Longitudinal section of the cranium

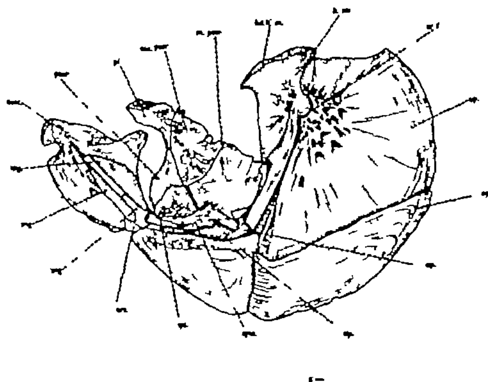
cl., claustrum *cnt. c.*, centrum of the second and third ertebra; *cnt 1 4 5* centrum of the first, fourth and fifth vertebrae *ep* epiotic; *l. eth.*, lateral thmoid; *myo* myodome; *n. a. 2. 3 4.*, neural arch of the second, third and fourth vertebrae; *sp* neural spine of second nd third vertebra; *n. sp. 4* neural spine of the fourth ertebra *oc sp* occipital spine *o.*, foramen for the lfactory nerve *orb sph.*, pleurosphenoid *pro.* prootic; *ph. pr* pharyngeal process of the basioccipital; *ps.* parasphenoid *rb. 4* rib of the fourth vertebra *oc* supraoccipital; *pter.* pterotic *vo* vomer

(Catla catla (Ham.)) Half

Longitudinal section of the cranium

l laustrum; *cnt. c.*, centrum of the second and third vertebra; *cnt. 1 4 5*, centrum of the first, fourth and fifth vertebrae *ep. o.*, epiotic; *eth.*, ethmoid; *ex. oc.*, exoccipital; *fen.*, fenstra; *f.*, frontal; *l. eth.* lateral ethmoid; *myo.* myodome *n. a. 2 3 4* neural arch of the second, third and fourth vertebrae *n. sp. c.*, neural spine of second and third vertebra *n. sp. 4* neural spine of the fourth vertebra; *oc. sp* occipital spine *o.*, foramen for the olfactory nerve *orb. sph.*, orbitosphenoid, *par.* parietal, *pl. sph.* pleurosphenoid; *pro.* prootic; *ph. pr.* pharyngeal process of the basioccipital *ps.* parasphenoid; *rb. 4* rib of the fourth vertebra *s. oc.*, supraoccipital, *sph.* sphenotic; *vo.*, vomer

Plate V



Inner View of the Opercular Series

ang. angular *ar. f.* articular facet *dent.*, dentary; *e. ptr.* ectopterygoid; *ent. ptr.* entopterygoid. h. m., hyomandibula *hd. h. m.* head of hyomandibula; *i. op.* interoperculum; *m. ptr.* metapterygoid; *op.* operculum *p. op.* preoperculum; *pl.* palatine; *qu.*, quadrate; *art.* retroarticular *sang.* supraangular *s. op.* suboperculum *sym.* symplectic

Catla catla (Ham.) Plate-II

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Bones of the skull-disarticulated

Fig (1) Supraoccipital; Fig. (2) Basioccipital Fig (3) Pterotic Fig (4) Prootic Fig (5)
Fig. (5) Epiotic Fig (6) Sphenotic; Fig. (7) Parietal Fig (8) Frontal Fig (12) Pterosphenoid
Exoccipital Fig (10) Vomer; Fig (11) Orbitosphenoid; Fig (16) Ethmoid
Fig. (13) Parasphenoid; Fig (14) Lacrymal; Fig (15) Supraorbital Fig (19) Suborbital second Fig
Fig. (17) Lateral ethmoid Fig (18) Suborbital First Fig (22) Nasal Fig (23) Supra-
(20) Suborbital third, Fig (21) Suborbital fourth Fig (22)
temporal; Fig (24) Rostral

ar.f. articular facet for hyomandibula a. s. l., anterior sinus lamella; b.l. ...
of the parasphenoid b. p. basal part; b.l. central body of the ethmoid; c.s.f. ethmoid cornu; f. ...
cavum sinus imparis cond. condyle; d. depression; eth. c. ethmoid cornu; ...
f. sac., fovea sacculi; gr. groove; l. pr., lateral process of the supraoccipital; oc. cond., occipital condyle
wing of the ethmoid m. p. main part; m. r., median ridge; ...

Catla catla (Ham.)

Plate-VII

Bones of the skull-disarticulated

Fig. (1) Palatine; Fig. (2) Ectopterygoid, Fig (3) Metapterygoid, Fig (4) Entopterygoid; Fig (5) Dentary; Fig (6) Angular; Fig (7) Maxilla; Fig (8) Premaxilla; Fig (9) Hyomandibula; Fig (10) Symplectic Fig (11) Quadrate; Fig. (12) Interoperculum; Fig. (13) Suboperculum Fig (14) Preoperculum; Fig. (15) Operculum.

ar. f. articular facet; *ar. hd.*, articular head; *cond.* condylar head of hyomandibula; *cond. hd.*, condylar head of palatine; *d. pr.* dorsal process of maxilla *gr.* groove; *hd.* head of hyomandibula; *l. pr.* lateral process of the premaxilla; *art.* retroarticul *r. pr.* rostral process of the maxilla; supraangular; symphysis; ventralar process of the maxilla

Catla catla (Ham.)

Bones of the skull-disarticulated

Fig (1) Supraoccipital Fig. (2) Basioccipital Fig (3) Pterotic Fig (4) Prootic Fig (5) Epiotic; Fig (6) Sphenotic Fig (7) Parietal Fig. (8) Frontal Fig (9) Exoccipital Fig (10) Vomer; Fig (11) Orbitosphenoid Fig (12) Pleurosphenoid Fig (13) Parasphenoid; Fig (14) Lacrymal Fig (15) Supraorbital Fig (16) Ethmoid Fig. (17) Lateral ethmoid; Fig (18) Suborbital First, Fig (19) Suborbital second Fig (20) Suborbital third Fig (21) Suborbital fourth Fig (22) Nasal Fig (23) temporal Fig (24) Rostral.

ar. f. articular facet for hyomandibula; a. s. l., atrium sinus imparis; c. bd., central body of the ethmoid; of the parasphenoid; b. p., basal part; c. s. l., cavum sinus imparis; cond. condyle; d., depression; eth. c., ethmoid cornu; f. sac., fovea sacculi; gr., groove; l. pr., lateral process of the supraoccipital; wing of the ethmoid; m. p., main part; m. r., median ridge; oc. cond., occipital condyle

EDTA, using Erichrome Black T indicator at pH 10 and calcium only was determined at pH more than 12 using Murexide potassium sulphate mixture magnesium was determined by difference Iron was determined by titrating the solution with EDTA using salicylic acid in methyl alcohol at pH 2 3 as indicator

It will be seen from the above account that nearly all methods in which EDTA is used as the titrant involve three operations for obtaining accurate results these are (i) the dissolution of limestone dolomite or any other calcareous material in hydrochloric acid or any other reagent (ii) the removal of iron and aluminium and (iii) the separation of calcium from magnesium or vice versa. These operations as well as the stages involved in the method of analysis prescribed by the Standard Institutions would take a long time to know the contents of calcium and magnesium carbonates in limestones or dolomite or any other calcareous material, which are the primary requirements of consumers. The method of Standard Institution is very good for the complete analysis of these materials and the methods involving the use of EDTA are equally efficient for the accurate determination of calcium and magnesium in them

Vogel[13] observed that freshly precipitated calcium carbonate dissolves readily in concentrated solution of ammonium chloride in the cold. Later Cantoni and Goguelia[14] found that calcium and magnesium carbonates react with ammonium chloride and the reaction goes to completion at boiling temperature. This observation has been confirmed by Singh[15] by the estimation of ammonia and carbon dioxide evolved on the completion of the reaction and comparing the experimental values with those theoretically expected according to the following chemical equation



$$CaCO_3 + 2NH_4Cl = CaCl_2 + 2NH + CO_2 + H_2O$$

The authors observed that calcium and magnesium carbonates in limestone dolomite and other calcareous materials also react with ammonium chloride in the same manner and the reaction goes to completion at boiling temperature in a short time and other metals present in these materials go into solution, if at all, in traces. Hence they have used this method for the extraction of calcium and magnesium from the aforesaid materials which is evidently quicker as the stage for the removal of iron and aluminium is automatically avoided by the peculiarity of the process of solution. The total calcium and magnesium in the ammonium chloride extract is estimated by titration with EDTA using Erichrome Black T indicator and calcium alone in the solution is determined, again by EDTA titration, using GbHA indicator The investigations conducted under the auspices of the Indian Standard Institution on the subject gives conclusive proof of the accurate estimation of calcium in presence of magnesium in ratios varying from 0 0 to 100 per cent in the mixture by the use of GbHA indicator provided the amount of magnesium is not more than 25 mg in 100 ml. of titre volume (private communication)

The details of the method followed by the authors are given below

EXPERIMENTAL

Reagents Used —

(1) A R ammonium chloride.

(2) M/100 disodium salt of EDTA solution obtained by dissolving 4 g of disodium salt of EDTA in a litre of redistilled water and standardising it with a standard calcium solution

(3) Ammonium buffer of pH 10 obtained by dissolving 67 g of ammonium chloride in 570 ml. of liquor ammonia and making upto one litre.

(4) 1% potassium cyanide solution.

(5) Triethanolamine.

(6) Absolute alcohol

(7) 4 / caustic soda solution obtained by dissolving 40 g caustic soda pallets in one litre of redistilled water

(8) Concentrated hydrochloric acid.

(9) M/100 standard calcium solution obtained by dissolving 1g calcium carbonate in hydrochloric acid and making upto one litre.

(10) Erichrome Black T indicator obtained by dissolving 0 2 g of the indicator in 15 ml. of triethanolamine and 5 ml. of absolute alcohol.

(11) Murexide—sodium chloride indicator obtained by mixing Murexide and sodium chloride in the ratio 1 100

(12) 0.25% Glyoxal bis Hydroxyanil (GbHA) indicator obtained by dissolving 0·25 g of GbHA in 100 ml of absolute alcohol.

Procedure

Limestone is finely powdered and sampled and then sieved through a 100 mesh sieve. 1 g of limestone is dissolved in necessary amount of ammonium chloride solution and the solution after filteration and washing is made up to 250 ml

Th total calcium and magnesium is determined by titration with EDTA at pH 10 using Erichrome Black T indicator Calcium in the mixture containing magnesium is estimated by titrating the mixture at pH more than 12 against EDTA using Murexide Indicator

In actual procedure 5 ml. of limestone solution in ammonium chloride are taken, 4-5 drops of triethanolamine solution and 4 5 drops of Erichrome Black T indicator are added to it. The solution is then heated to about 60°C. To maintain the pH 10 ammonium buffer is added to the solution which is titrated against a standard solution of *EDTA* The volume of EDTA consumed gives the value of total calcium and magnesium present in the solution.

Another 5 ml. of limestone solution are taken and treated with triethanolamine in the same manner as before. To this are added 15 ml. of 4% caustic soda solution to maintain the pH more than 12 and about 0·01-0·05 g of Murexide-sodium chloride indicator The solution is then titrated against standard EDTA. The titre gives the amount of calcium alone in the solution The difference between the first and the second determinations gives the amount of magnesium in the original solution. It was however found that the results obtained by the use of this indicator are not very accurate and repeatable as the end point is not very sharp that is the same end point is not obtained every time these results are not given in this paper

The amount of calcium in the solution of limestone was hence determined by using more recent Glyoxal-bis Hydroxyanil indicator (GbHA) which has been described by Goldstein[16] for complexometric titration of calcium and used by Verma and Bhuchar[17] for the estimation of calcium by EDTA method. GbHA gives a red coloured calcium complex at pH more than 12 which is soluble in alcohol. For this determination 5 ml of the limestone solution in ammonium chloride containing calcium and magnesium are taken. 4 drops of triethanolamine (to mask heavy metals) a known excess of standard EDTA solution 2·5 ml of alcohol, 15 ml. of caustic soda solution to bring the pH to more than 12 and 5-10 drops of GbHA solution are added to it. The solution is then titrated against standard calcium solution The colour of the indicator changes from yellow to orange and finally to pink due to the formation of calcium complex with GbHA The end point is quite easy to perceive and sharp Magnesium is then determined by difference.

Magnesium has also been determined directly after the estimation of calcium by the aforesaid method, by adding concentrated hydrochloric acid to destroy the calcium complex with GbHA and again adding the masking agents, and titrating it against standard EDTA solution at pH 10 maintained by adding ammonium buffer using Erichrome Black T indicator The EDTA consumed directly gives the value for magnesium (private communication)

The results obtained are given in Table in which the 2nd and 3rd columns give the name and source of the mineral the 4th and 5th columns give the percentage of calcium and magnesium in the minerals as determined by the usual classical method the 6th and 7th columns give the percentage of calcium and magnesium as determined by EDTA method, using Erichrome Black T and GbHA indicators, from HCl solutions made for experiments used for estimations by the classical method the 8th and 9th columns give the percentage of calcium and magnesium obtained from the solutions made in ammonium chloride solution

DISCUSSION

It will be seen from the Table that very accurate and reliable percentages of calcium and magnesium can be obtained by the method suggested by the authors in a comparatively short time. The method involves only two operations —

(i) the dissolution of the mineral containing calcium and magnesium carbonates in ammonium chloride, and

(ii) titrating the solution obtained against EDTA in two stages using (a) Erichrome Black T and (b) GbHA indicators.

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TABLE

Sl N	Kind of Stone	Locality	By th old method (Gravimetrically)		Dissolving the sample i HCl and titrating against EDTA		Dissolving the sample in ammonium chloride & titrating it against EDTA	
			% $CaCO_3$	% $MgCO_3$	% $CaCO_3$	% $MgCO_3$	% $CaCO_3$	% $MgCO_3$
1	Limestone	Dehra Dun	96.20	2.40	96.0	2.30	96.5	2.32
2	Dolomatic Marble	Raj Nagar	94.52	4.12	95.0	4.40	95.0	4.20
3	Limestone	Monda	94.80	5.20	91.0	4.80	91.0	5.01
4	Limestone	Issued by — Middles Bourgh	65.90	32.80	66.5	31.70	66.8	31.50
5	Marble (1)	Makarana	96.30	1.60	97.0	1.80	96.5	1.63
6	Marble (2)	Makarana	76.80	18.28	80.0	19.00	80.0	18.50
7	Limestone	Katni			95.0	1.24	95.5	1.26
8	Limestone	Kallaras			74.0	3.28	74.0	3.28
9	Limestone				32.0	2.10	52.5	2.10
10	Limestone				78.5	3.28	78.5	3.20
11	Limestone				73.5	2.94	74.0	2.52
12	Limestone				81.5	2.5	81.0	2.52
13	Limestone				73.5	2.94	73.5	2.94

CONTENTS

THE ORIGIN OF LIFE

O N PERTI
Professor of Chemistry Th. D.S.B Government College Naini Tal India

SUMMARY

The problem of the origin of life has been critically evaluated. It has been pointed out that the only kind of life with which we are familiar is life as found on the Earth and the discussion should be confined to the terrestrial form of life. The latest evidence available regarding the hypothesis of extra-terrestrial origin of life has been reviewed It has been pointed out that the organized microstructures found in carbonaceous chondrites can possibly be obtained in the laboratory Experimental production of such structures has been described

In support of the hypothesis of terrestrial origin of life the role of photosynthesis of amino acids and peptides in aqueous solutions has been emphasised

The problem of the origin of the first cell like structure has been discussed. The improbability of the suggestion that the constituents of the cell such as nucleic acids adenosine phosphates enzymes etc. were obtained by Nature separately and then brought together to form the first animal cell has been stressed. The recent theory of Bahadur and Ranganayaki regarding the origin of cell has been elaborated. It has been pointed out that the property of duplication and adaptability to environment can be manifested by inanimate matter under suitable conditions Experimental preparation of approximately cell-size units capable of growth, multiplication and metabolic activity has been described. It has been suggested that the first cell might have evolved in this manner A suggestion has been made that the manifestation of biological properties of a higher order by inanimate matter is merely a question of arranging the inanimate matter in such a way that it can manifest its inherent properties of replication and adaptability in a harmonious manner

INTRODUCTION

The problem of the origin of life intrigued philosophers since the most remote antiquity In different ages different answers were given. [1] Thinkers or religious teachers in ancient China, Egypt and Babylon believed in various traditions and legends all of which go to show that the origin of life was a creative act of God at some remote antiquity [2] The ancient Hindus were generally of the opinion that life originated by natural means through the interplay of what were believed to be the primary elements [4] They also

pointed out that initially life originated in water.[3] The philosophers of the Ionian School (600 B. C.) in Greece taught that animal life originated in sea slime by the action of heat, sun and air.[4] The views of Aristotle (384-322 B. C.) deserve special mention as they had very wide ramifications and influenced human thought for the next one thousand years or more. Aristotle taught that living as well as non living things were produced by the union of *matter* with *form* which is the *entelechy* or *soul* of living things. It has been suggested that Aristotle's theory of spontaneous generation of life was accepted by Romans and through them by the founders of Christianity in particular by Basilius and Saint Augustine who believed that this phenomenon of spontaneous generation of living things was the manifestation of the will of God.[5] The ideas of the Christian church dominated European thought for the next ten centuries or more. Mention may however be made of the theory of Alexander Neckam (1157 1217) who believed that goose can be produced from fir trees with the help of salt from sea water[1] of the reports of travellers like Odorico da Pordenone (1331) Maundeville (1300-1372) and others who talked of having seen in the Orient lambs obtained from melon like fruits of some trees[8] and of the recipe of Paracelsus (1493-1541) for the preparation of *homunculus* or the embryo of the little man who required human blood for its nourishment.[9] Many prominent scientists of the sixteenth and seventeenth century had also similar views. Van Helmont (1577 1644) gave a prescription for obtaining mice from wheat kernels.[10] Harvey (1578-1657) who coined the famous phrase *Omne vivum ex ovo* (all living from the egg) believed in *generatio aequivoca* (spontaneous generation) of worms, insects etc.[11] Even Descartes (1596-1650) and Newton (1643-1727) accepted without reserve the theory of spontaneous generation of life from lifeless matter.

The first available mild contradiction of the theory of spontaneous generation is to be found in the treatise of Francesco Redi entitled *Esperienze intorno alla generazione degl'insetti* published in 1668. His work attracted little attention as he could not pick up enough courage to denounce the theory outright. In the meantime Leeuwenhoek (1632 1723) used microscope and discovered a new world of *viva animalcula* the tiny little beasts.[12] The work of Leeuwenhoek Joblot[13] Buffon (1707 1788)[14] Needham (1713-1781)[15] and others of the same period indicated that in fermentation in the decomposition of vegetable and animal matter living microorganisms were produced by something which got into them from air. They however continued to have faith in the spontaneous generation theory and generally believed that in every microscopic particle of organic matter there was a *vital force* or *vital principle* which could give rise to life. Even Lamarck (1744-1829) in [illegible] his general concepts of Nature continued to believe in the position [illegible] spontaneous generation of mushrooms and parasites.[16] In 1765 L. Spallanzani published some experimental results which questioned the theory of spontaneous generation of life.[16] However his work failed to convince many. In 1859 Pouchet[17, 18] published a seven hundred page account of the [illegible]

ments indicating the possibility of spontaneous generation and The French Academy of Sciences offered a prize for experimental confirmation or disconfirmation of the theory of autogeneration of living things This prize was awarded to Louis Pasteur Pasteur showed by a series of brilliant experiments[19 to 21 22] what was believed to be a conclusive demonstration that living systems could not arise out of the non-living material. He showed that only life begets life and that life cannot be created spontaneously out of the non-living Though some scientists foremost amongst them being Bastion[23] questioned the experiments of Pasteur but a vast majority of scientific workers unequivocally accepted his findings This gave a sort of death blow to the theory of spontaneous generation and many scientists jumped to the conclusion that spontaneous generation of life could never have occurred Majority of the scientists were of the opinion that the problem of the origin of life cannot be tackled by scientific methods and must be considered to be unworthy of the attention of any serious scientific investigator

Even up to the middle of the present century one can find only a very few isolated attempts towards a scientific solution of the question of the origin of animal life. Comparatively recent developments in the field of chemistry biochemistry, and particularly in the sphere of knowledge about the primitive Earth and its atmosphere has brought about a change in this attitude. Scientists have begun to realise that Pasteur was working in the time-scale of his life and was not considering what might have happened 2 3 thousand million years ago[24] The recent event which focussed the attention of scientists on to this problem was the holding of an International Symposium on this subject at Moscow on 19th 24th August, 1957 In his *Introductory Address* at this symposium Academician A. I Oparin remarked twenty or thirty years ago, even the calling of such a Symposium would have been completely impossible in that, until quite recently experimental scientists had not paid sufficient attention to this problem '[25] Since then the problem of the origin of life is considered to be a very fascinating and complex problem It poses questions bristling with complexities and the modern scientific investigator is not averse to accept the challange to investigate it experimentally

Some Fundamental Considerations

N W Pirie has emphasized that in any discussion relating to the problem of the origin of life there is no place for any kind of dogmatism howsoever supported it may be by experimental or other kind of evidence[26 27] There is always more than one possibility and one cannot afford to ignore another point of view or another aspect of the problem at any stage

The only kind of life with which we are familiar is the life found on our planet called the Earth This statement does not assume that life on other planets in the universe does not exist today or has never existed there in the

past During comparatively a short time human beings are now able to send astronaut after astronaut into space. Why can we not assume that 2 3 thousand million years ago intelligent beings might have lived on other planets and might have developed space travel ? It has recently been pointed out that intelligent beings on other planets might still be trying to contact us by radio and a search is being currently conducted by the U S National Radio Astronomy Observatory for interstellar communications of this type.[17,18,19] Up to the present, as far as is known to me, no positive evidence has been obtained but such a possibility is no longer considered to be unscientific.[20] My contention is that up to the present the only kind of life with which we are familiar is the one found on the Earth and it would be desirable to restrict oneself to the kind of life found on this planet only

There is considerable scientific evidence to believe that our planet has a finite age which is of the order of 4-5 thousand million years.[21,22,23] We may well ask whether this planet always possessed living organisms or life made its appearance sometime after its formation. Preyer in 1880 developed a very cogent theory of the eternity of life.[24] His main argument was that if life cannot be created out of the non-living then it must have existed ever since the Earth was formed in the form of *universal life essence* This theory had its adherents for sometime but was later given up in the face of newer discoveries and today it is only of historic interest. A cording to H C. Urey the earlier stages in the formation of the Earth involved temperatures upto 2000°C, a temperature at which even if living organisms existed they would be completely destroyed.[26,27,28] It is highly probable that life made its first appearance on the Earth 2 5 thousand million years ago as that is the probable age of the oldest detectable fossils.[29] Other considerations such as the conditions essential for existence of life, also point to near about the same period for the appearance of life on the Earth.[30] The available evidence however is not conclusive but from all that we know today it is highly probable that life made its first appearance on this planet a couple of thousand million years after its formation from the primeval nebula from which it condensed.

The question that now arises is whether the first living organisms were formed here on the Earth or it was brought to our planet from elsewhere in space. We cannot dismiss either hypothesis as ridiculous or unscientific. If we accept the hypothesis of extra terrestrial origin of life on the Earth then we accept the ready packaged solution of the problem On the other hand if we have faith in the terrestrial origin of life then we have to explain the multiple mechanisms which might have taken place before life emerged from the non living Scientific evidence is now available in partial support of either hypothesis

THE HYPOTHESIS OF EXTRA TERRESTRIAL ORIGIN OF LIFE

In any consideration of the hypothesis of the extra-terrestrial origin of life on the Earth mention must be made of the earlier theories dealing ...

what have been termed as *cosmozoa* or *panspermia* since more or less similar ideas are present even today. In 1865 Richter made the suggestion that somewhere in the universe there were always cosmic bodies present on which life existed in cellular form.[10] Fragments from these could float in the interstellar space and could be accidentally brought into contact with a planet where conditions for life were already favourable. Liebig also held similar views and believed in eternal organic life which was never really created but was merely transmitted from one planet to the next.[11] In 1884 Helmholtz made the suggestion that life germs were brought to the Earth by meteorites.[12] In the beginning of the present century Arrhenius first advocated this idea in all seriousness in his book *Lehrbuch der Kosmischen Physik* (1903). He proposed that the spores of microorganisms (*panspermia*) could be carried into upper atmosphere and from thence they could escape into interplanetary space and given sufficient time, guided by the pressure of sunlight, they could land on another planet or elsewhere in the universe.[13] Given favourable conditions and sufficient time they could start a chain of events which would ultimately result in various life forms that we see today.

There is scientific evidence to show that microorganisms do exist in the upper atmosphere[14] and that bacterial spores can survive considerable temperature variations and can resist complete lack of nutrients for considerable periods of time.[15] There is however no direct evidence available for the existence or non-existence of microorganisms in space. It is also unlikely that bacterial spores can survive the onslaught of ultraviolet and other radiations which are present in interplanetary space.[16] The suggestion of Helmholtz that meteorites can be their possible carriers has recently been revived. Currently critical examination of meteorites is being carried out from the standpoint of possible biological significance. It may here be recalled that the earlier investigations of Pasteur[17] and others[18] gave negative results but studies of Lipman[19,20] during 1932-36 revealed the presence of a number of common bacterial species from the centres of surface sterilised meteorites. The results of Lipman are however not unambiguous as they may be due to terrestrial contamination of the meteorite after its fall. The recent chemical and physical studies of meteorites are of greater interest. Presence of sulphur compound[21] and quite a lot of unidentified organic compounds has been reported. Particular significance is attached to the report of Prof. M. Calvin of the University of California, in 1957 who has claimed to have found an organic compound having spectral and solvent properties closely resembling cytosine, one of the basic units of which the nucleic acid molecule is built.[22] Upto date studies show that amongst the compounds which have been definitely identified are hydrocarbons (paraffins+naphthenes+aromatics), fatty acids, aromatic acids and phenols of low molecular weight and amino acids.[1] Kaplan *et al* have recently presented detailed analyses of several meteorites and have reported the presence of considerable amounts of amino acids.[23] Amongst the amino acids detected are arginine, ornithine, lysine, histidine, aspartic acid, glutamic acid,

glycine α-alanine, β-alanine serine proline valine, threonine, leucine, tyrosine, phenyl alanine and methionine. It is interesting to note that a mixture of many of these amino acids in different meteorites has been found. Studies based on radioactive carbon[56, 57] indicate though not unambiguously[58] that some of the meteoritic carbon is of biogenic origin. Special significance attaches to the recent reports of the presence of organised micro-structures found in some carbonaceous chondrites by Dr George Claus of New York University Medical Centre and Prof Bartholomew Nagy of the Department of Chemistry Fordham University New York.[59] They have made the suggestion that the organised elements may be micro-fossils indigenous to the meteorites. Studies of other workers [60-64] has given support to this view though some are of the opinion[65] that these micro-structures may merely be the result of contamination from terrestrial sources. On the available evidence it is impossible to decide on the exact nature of these 'organised elements'.[66] If the organised elements should prove to be indigenous microfossils then it may lead to the formulation of either of the two following hypotheses (i) Life is not a unique property confined to the planet Earth but it may have evolved in various parts of the universe[44] or (ii) These organised elements are terrestrial forms that contaminated the moon from the Earth during early geological times[47] by the impact of meteorites into terrestrial bodies of water

To sum up the main evidence available today for the hypothesis of extra-terrestrial origin of life we may say that it is unlikely that unprotected life bearing spores could travel in space and be not destroyed by intense radiations present there. If the contention of Claus and Nagy is tenable then it is possible to imagine that these spores could have been brought to the Earth by meteorites. Lastly there is the suggestion that contact with the Earth was made by intelligent beings living on other planets. So far as the question of contamination by space travelling extra-terrestrial intelligences is concerned future alone can confirm or disconfirm the hypothesis. At the present the hypothesis of extra terrestrial intelligences in the words of Dr Briggs[69] 'is as likely as any other for are we not taking precautions to avoid contamination of other planets by man made satellites.[69,70]

THE HYPOTHESIS OF TERRESTRIAL ORIGIN OF LIFE

I would now take up the hypothesis of terrestrial origin of life. We have to make a beginning by assuming the correctness of the uniformitarian principle or the principle of Lyell[70] This principle substantially means that no occult phenomena are involved and no forces or processes operated to bring life into being in the past that do not operate now. This means that we have to make a beginning by accepting the theory of abiogenesis that is life originated from non life It may be pointed out here that the assumption of the uniformitarian principle may not be wholly valid as Haldane has argued[71, 72] that physics and chemistry in the pre-cambrian era may

have been significantly different from what we have today Nevertheless one has to make a start by accepting the uniformitarian principle otherwise further progress in scientific investigation of the origin of life on this Earth would be practically impossible.

Having accepted the uniformitarian principle it follows that we have to search in the present world for processes which might have occurred 2 3 thousand million years ago In this connection the ideas of Henderson given in *Fitness of The Environment* are very important. He points out that in any consideration of *biopoesis* or life making we must take into account the gases present in the primitive atmosphere and the chemical elements present in Earth's crust and their chemical and physical properties.

There are reasons to believe that the atmosphere of the Earth has gradually evolved.[73] The work of Urey[74] Vinogradov [5] Sokolov[76] and others tells us that in the first stages of the Earth the atmosphere consisted mainly of hydrogen and helium together with small amounts of neon Later nitrogen, carbon monoxide, carbon dioxide methane, ammonia water vapour and sulphuretted hydrogen were added to it along with traces of other rare gases. There was practically no oxygen which appears to have been added at a much later biogenic stage Thus the prebiogenic atmosphere of the Earth was highly reducing in its chemical nature.[77] There were a variety of energy sources such as sunlight, lightening terrestrial radioactivity volcanic heat etc. which were continuously acting on the primitive atmosphere. It is obvious that a variety of reactions could take place. In the consideration of the origin of life we would be primarily concerned with the production of organic compounds out of which the most important ones would be amino acids which are the building units of proteins the basic stuff of animal life.

ORIGIN OF AMINO ACIDS

W Groth[78 79] and also A N Terenin[80] have shown experimentally that a number of organic compounds can be produced from gaseous mixtures constituting the primitive atmosphere by exposing them to ultraviolet radiations. Others [81] have been able to get more or less similar results repeating the experiments but using X rays and high energy electrons. Miller and Urey in U.S.A.[82-86] and Pavlovskaya and Pasynskii[87] in U S S.R and others[88-90] have tried to simulate conditions of lightening by passing electric discharge through a mixture of some of the gases present in the prebiological atmosphere of the Earth They could detect the formation of several organic compounds including amino acids. Table 1 gives a summary of the data.[77 90] Amino acids have also been obtained by thermal processes.[90 91]

Krishna Bahadur and co-workers have published results on the photochemical formation of amino acids in aqueous medium using paraformaldehyde as source of carbon [92-95] Paraformaldehyde is a compound of high mole-

cular weight which is produced by evaporation of an aqueous solution of formaldehyde known commonly as formalin It is known that carbon dioxide dissolved in water gets converted into formaldehyde when exposed to light.[93]

TABLE 1[99,77]

Products and yields of sparking a mixture of methane, ammonia, hydrogen and water vapor 710 mg of carbon added as methane

Compound	*Yield* (moles x 10^5)
Glycine	63
Sarcosine (methyl glycine)	5
Alanine	34
Beta alanine	15
N-methyl-α-alanine	1
α Amino-n-butyric acid	3
α Amino-*iso*-butyric acid	0·1
Aspartic acid	0·4
Glutamic acid	0·6
Imino diacetic acid	5·5
Urea	2
N-methyl urea	1·5
Glycollic acid	56
Lactic acid	31
α hydroxy butyric acid	0·1
Succinic acid	4
Formic acid	233
Acetic acid	15
Propionic acid	13

This indicates that paraformaldehyde could have been easily available in the prebiogenic state of the Earth Oparin[100] has quoted a private communication from A. Bach who found that if a mixture of formaldehyde and potassium cyanide in solution is kept aside then after a lapse of time a peptone like substance can be isolated from the mixture It is also interesting to recall that in the electric discharge experiments (Table 1) by far the greatest amount of organic substance produced is formic acid which in the reducing atmosphere of the primitive Earth could have been easily converted into formaldehyde which might have yielded paraformaldehyde. Whatever might have been the reason for the choice of paraformaldehyde as source of carbon in these experiments the results were very interesting. These workers found that under sterilised conditions a mixture of paraformaldehyde potassium nitrate [illegible] chloride and water when exposed to light from a 500 watt bulb could give rise to amino acids Depending upon the pH of the solution different amino acids were seen to be formed in the solutions. Later these workers found that [illegible] a mixture of paraformaldehyde, colloidal molybdenum oxide and water [illegible] produce amino acids with the help of light from a 500 watt bulb. [illegible]

experiments go to show that for the production of amino acids all that we need is water a carbon source some minerals, nitrogen of the air and sunlight.

I may mention here that some experiments recently carried out in my laboratory lend support to the view that the production of amino acids by fixing nitrogen of the air in aqueous solution is not a very difficult process. For instance, it is well known that tartaric acid decomposes rapidly when its solution is exposed to ultraviolet light.[181 184] In this process a lot of energy is liberated and it is quite possible that fixation of the nitrogen of air can take place along with decomposition G. B. Sharma was asked to reinvestigate the decomposition of tartaric acid under ultraviolet light. He was able to detect the presence of nitrogen in aqueous solutions of tartaric acid kept under a source of ultraviolet radiations Chromatographic analysis revealed a couple of ninhydrin positive spots. Sharma secured a stipend and left my laboratory to work at Biochemistry Department of A. & M College, Texas. This work was again taken up in a more systematic manner by (Miss) Vimal Paul. She started with chromatographically pure tartaric acid and used nitrogen free double distilled water for preparing solutions She exposed a 5 % aqueous solution of tartaric acid to ultraviolet radiations from a 500 watt mercury vapour quartz lamp When the solution was kept at a distance of 38 cms. from the lamp, she found after 1 hour of exposure the presence of glycine alanine and even glutamic acid in the solution. When exposure was continued upto 4 hours further ninhydrin positive spots were detectable in the developed chromatogram, two of them possibly corresponding with α-amino butyric acid and valine The work is being persued further and citric acid is also being tried on similar lines. At present it is not possible to say whether the amino acids appear in solution one by one or in the beginning only one is formed and gets converted into others with the help of several free radicals which might be expected to be present in the solution The problem and experiments relating to Interconversion of amino acids would be described in some detail in the next section.

It appears that in the beginning lightening, sunlight (containing more ultraviolet than at present) terrestrial radioactive radiations and volcanic heat made the gases of the primitive atmosphere react to produce certain organic compounds including a small amount of amino acids. These compounds got dissolved in water which also contained many minerals. When sunlight played on such solutions the nitrogen of the air could be constantly converted into different amino acids and thus the ingredients required for the production of proteins were made available in quantity There is little doubt that the bulk production of amino acids must have taken place in aqueous medium using simple chemicals and sunlight as energy source.

Inter Conversion of Amino Acids and Production of Peptides in the Prebiological Era

The photochemical formation of different amino acids and peptides in aqueous medium has been observed by Krishna Bahadur *et al* [108 184] and also

by us.[103 106,107,108] Our starting point was chromatographically pure amino acids. To some of them we added sugar as energy material. To others we added colloidal metallic oxides like those of molybdenum, vanadium or iron. All these solutions were perfectly sterilised and then exposed under sterilised conditions to sunlight to light from a 1000 watt bulb and in certain cases to ultraviolet light. We noticed that chemical changes occurred only in those solutions which were exposed to light but similar solutions kept in dark or wrapped in heavy black cloth remained uneffected. Exposure to different kind of light upto 600 hours duration were carried out and periodically the solutions were taken out under sterilised conditions and examined by the chromatographic method of analysis. To our surprise we noted that the solutions of single amino acids could give rise to other amino acids. At the same time we could also notice that these amino acids were combining amongst themselves to yield peptides. As the time of exposure was increased a bewildering number of compounds were appearing to be formed. In fact, we found that we could not positively identify many of them and had to content ours lves by mentioning in our papers[illegible] ... plus several unidentified products."

Recently (Miss) Vimal Paul has carried out very interesting studies on aliphatic amino acids by exposing their aqueous solutions to ultraviolet light. To explain the importance of this study it is necessary to recall some earlier work. The splitting of ammonia from amino acids or deamination of amino acids has been observed by various workers when amino acid solutions are exposed to radiations such as ultraviolet, λ-rays, cathode rays, radioactive radiation from radon etc.[109,110 111,112,113,114] Lieben and [illegible] have pointed out that only the α am no group undergoes this reaction.[115] In the case of glycine it has been suggested that ammonia and glycollic acid are formed [116] A vide variety of amino acids have been found to be readily deaminated.[117 118] Evolution of ammonia has been observed in the case of alanine [119] We have found earlier that aqueous solutions of amino acids undergo complicated changes when exposed to sunlight or to light from a 1000 watt bulb [100–103] In the case of sunlight it was noticed that the rate of reaction was slightly higher when transparent quartz containers were used. Moreover these experiments were being conducted at Naini Tal at an altitude of about 7000 ft. All this pointed to the role of ultraviolet flux in sunlight. It occurred to us that in the previously known deamination studies stronger sources of radiation were used and prolonged exposures were given with a view to detect the formation of ammonia It was just likely that if short exposures and mild sources of radiat ons are used then the photolysis may initially proceed in a different direction

Miss Vimal Paul has recently found that if we use 0.1 solution of glycine and expose it for very short periods to ultraviolet radiations from a 500 watt mercury quartz lamp interesting changes are observable. After 1 minute of exposure alanine is detectable in the solution When the expo

time is 2 minutes glycyl-glycine amino butyric acid and valine are also detectable. There was a faint indication of the formation of glycyl-glycyl glycine also. As the exposure time was increased to 30 minutes or more the peptides were found to disappear and only the presence of alanine was traceable. Curiously enough when a similar solution of alanine was used the formation of glycine could be detected after an exposure of 30 seconds. When exposure was continued upto 5 minutes presence of peptides was also traceable. However on further exposure the chromatographic analysis of the solution showed only the presence of alanine These studies indicate that alanine can be converted into glycine or vice-versa if we use very short exposures to ultraviolet. Even formation of peptides is possible under these circumstances.

I may also mention some of the very recent experiments carried out in my laboratory A solution of glycine (0 1g) alanine (0 1g) and ascorbic acid (0 1g) in 100 ml water was exposed to ultraviolet light. After an exposure of 30-90 seconds several compounds could be identified in the solution. Glycine, alanine valine, amino butyric acid, diglycine, triglycine tetraglycine, penta glycine, alanyl-alanine alanyl-glycyl-glycine could be detected. On continuing exposures upto 15 minutes the quantity of these substances appeared to increase as judged by the intensity of spots on the chromatograms When exposures were carried out beyond 15 minutes, it was noticed that the intensity of spots on the chromatograms was diminishing indicating that the quantity of these substances formed is also diminishing

The above experiments were carried out by exposing naked solutions to ultraviolet radiations from a mercury quartz lamp kept at a distance of about 50 inches. R. C. Rastogi has carried out similar experiments using a richer source of radiations—a naked carbon arc (55 volts, 6-7 amperes) He used sterilised aqueous solutions enclosed in transparent quartz vessels Some of the results obtained by him are given below:

Exposure of a sterilised aqueous solution of glycine kept at a distance of 15 cm. from the carbon arc showed the formation of alanine in 5 seconds. An exposure of five seconds was given and the solution was analysed chromatographically the next day When the exposure time was increased further the chromatographic analysis indicated the disappearance of alanine. The experiment was repeated with the addition of ascorbic acid to glycine solution. After an exposure of 5 seconds very small amounts of alanine, valine, glycyl-glycine could be detected. When exposure time was increased upto 2 minutes more amino acids and peptides were seen to be formed. Further increase of exposure time particularly beyond five minutes indicated decompositions of the products formed.

On exposure of alanine solution to carbon arc the formation of glycine could be detected after 40 seconds. However when the exposure time was increased further the glycine formed presumably decomposed. In this expe-

riment the distance between the flask and the carbon arc was 15 cm. In order to have still milder conditions the distance was next increased to 45 cm. Glycine could then be detected after 10 seconds of exposure time. After 30 seconds of exposure even alanyl-glycyl-glycine and glycyl-glycyl-glycyl-glycine could be detected. If exposure time is further increased then the reaction follows the course of decomposition. When a mixture of glycine and alanine was used however no new compound was detectable after exposure of 5 second to 10 minutes. It appears that under these circumstances only inter-conversion of glycine to alanine and vice-versa takes place. If ascorbic acid is added to the mixture of glycine and alanine then after 5 seconds of exposure formation of valine could be detected. Increase of exposure time upto 2 minutes indicated formation of newer amino acids and even peptides. Further exposure, however led to the decomposition path.

These and similar studies would shortly be published by us in greater detail. They point to a way that many amino acids can be obtained by a single amino acid merely by the agency of light. Our investigations with tyrosine have been very extensive and thorough. These investigations have been partly incorporated in the Ph. D. thesis of H.D. Pathak.[10a] Some of these results have already been published [10b] and others which would be published shortly in detail are briefly described here as they are of interest in connection with the problem of the origin of life.

It was found that when an aqueous solution (sterilised) of tyrosine was exposed to sunlight phenylalanine was formed after about 80 hours of exposure. 20 hours further exposure showed the formation of glycine. When exposure was continued upto 400 hours glycyl tyrosine could also be detected. Besides a few more compounds were also formed which could not be identified. A very faint spot of dityrosine appeared after 500 hours of exposure on the paper chromatogram but it disappeared soon.

If to tyrosine solution colloidal molybdenum oxide was added and the mixture was irradiated with sunlight phenylalanine formation could be detected on 80 hours exposure. Glycine was produced on 100 hours exposure. Further exposure upto 300 hours caused synthesis of glycyl-tyrosine. When exposure was continued for 100 hours further alanine could also be identified in the solution. A few more compounds were also formed which could not be identified positively.

An identical solution of tyrosine containing colloidal vanadium per-oxide indicated the formation of phenyl alanine on 80 hours exposure and glycine on 100 hours exposure to sunlight. Glycyl tyrosine was found to be formed on 250 hours irradiation with sunlight. When the solution was ex-posed to sunlight upto 400 hours, in addition to alanine glycyl-alanine was also found in the solution. A few other compounds were also detected in traces but they could not be identified positively by us. It appears

bable that vanadium pentoxide helps in the formation of glycyl-alanine (cf. when molybdenum oxide is used)

When colloidal ferric oxide was used in place of molybdenum or vanadium oxides slightly different results were obtained. Phenyl alanine was found on 80 hours exposure. Further 20 hours irradiation caused the production of alanine and glycine If exposure was continued upto 250 hours the solution also indicated the formation glycyl tyrosine 100 hours further exposure showed the presence of glutamic acid also There were also other products in traces It appears that colloidal ferric oxide is perhaps more effective than either colloidal molybdenum oxide or vanadium pentoxide in catalysing this type of photochemical change.

Next in the above type of experiments sunlight was replaced by ultra violet light from a 500 watt high pressure mercury vapour quartz lamp for reasons which have been mentioned previously in this section It was found that photolysis of tyrosine and photo-synthesis of peptides in tyrosine solution was more pronounced.

When sterilised aqueous solution of tyrosine in transparent quartz vessels was exposed to ultraviolet light alanine was seen to be formed on 3 hours exposure. Further exposure of 3 hours showed the formation of glycine and phenyl alanine. When exposure time was increased further by 4 hours glycyl-alanine and glycyl tyrosine could also be detected in the solution. Irradiation upto 15 hours exposure time indicated the formation of glutamic acid also

When the solution contained colloidal molybdenum oxide, formation of alanine could be detected in 2 hours exposure time Exposure upto 6 hours indicated the formation of glycine and glycyl-tyrosine in addition to alanine. Further exposure of 4 hours showed the presence of diglycine in the solution On increasing the exposure time further glutamic acid could also be identified in the solution Formation of phenyl-alanine, however could not be detected.

More or less similar results were obtained if colloidal molybdenum oxide was substituted by colloidal vanadium pentoxide. Not much difference was noticeable if colloidal ferric oxide was substituted in place of colloidal molybdenum oxide or vanadium pentoxide

From these experiments it appears probable that ultraviolet light is more effective in the photolysis of tyrosine and photosynthesis of peptides. Photocatalysts such as colloidal oxides of iron vanadium or molybdenum only appear to slightly accelerate the photochemical changes. It may be mentioned here that though ultraviolet light produced rapid changes the radiations available in sunlight or in the light of a 1000 watt bulb are also quite effective. In the case of tyrosine and other aromatic amino acids there are quite rapid changes even when the source of light is the sun. In the case of tyro-

sine described here, in all cases it was noticed that the solution darkens and acquires a brownish colour Moreover as the exposure time increased the concentration of tyrosine was found to diminish in the solution.

Experiments were next designed to study the action of ultraviolet light on sterilised aqueous solutions of tyrosine with or without inorganic catalysts under nitrogen atmosphere between the temperature range 18-20°

In the case of pure tyrosine solution formation of glycine, glutamic acid, diglycine, glycyl tyrosine, phenyl alanine, proline, dityrosine, alanine and serine was noticeable Similar solutions of tyrosine containing colloidal molybdenum, vanadium or iron oxide gave more or less similar results.

It is possible to build up a whole scheme for the production of different amino acids from formaldehyde and glycine as has been suggested by Krishna Bahadur[121] There is little doubt that in solutions of amino acids studied by us a free radical mechanism is operative as has been suggested by us[122] Whatever may be the mechanism it is clear that one amino acid can give rise to other amino acids by the agency of light. There is little doubt that the nitrogen of the atmosphere plays a very significant role in this process. Further alongwith the production of other amino acids from one amino acid the formation of peptides is also noticeable. Thus no extraordinary conditions are required either for the production of amino acids or for the production of peptides. Moreover these experiments also suggest that how from one amino acid several other amino acids might have been obtained paving a way for a complex combination of amino acids as a natural sequence in the chemical evolution.

Origin of Fore proteins or Proteinoids in the Prebiological Era

It has been suggested by S W Fox[123] that proteinoids were first obtained from amino acids by a thermal process Fox and coworkers have shown[illegible] that if you heat anhydrous mixtures of amino acids upto 160 170° quite large protein type of molecules are produced having molecular weight in certain cases upto 20 000 The reaction has also been found to proceed in presence of strong phosphoric acid. It is on this basis that Fox has made the suggestion of a thermal pathway for the synthesis of proteins in the prebiological era.

There is little doubt that reactions of this type might have occurred on the primitive Earth. The sides of volcanoes and other thermal regions could provide temperatures of the order of 160-170° It is however difficult to imagine how such a process could produce a large amount of fore-proteins as nature could not possibly arrange for laboratory controlled temperature conditions Further such a type of synthesis of proteins however is unable to account for the origin of enzymes—the proteins with catalytic activity It is well known that cellular proteins are of two types

viz. structural or enzymatic. In all probability structural proteins possess no catalytic activity It is unquestionable that in any consideration of the origin of life the most important type of protein is the enzymatic protein as life as known to us on this Earth is unthinkable if we exclude the existence of enzymes If proteins arose by the thermal polymerisation how are we going to account for the fact that almost all enzymes that have been studied so far are irreversibly inactivated by heat These facts naturally raise considerable doubt about the role of thermal polymerisation of amino acids in the origin of proteins The value of thermal pathway can only be considered if it can be shown decisively or even in a general manner whether this thermal protein was incorporated into the first terrestrial organism. At present it does not seem possible to solve this problem. The role of thermal polymerisation of amino acids must therefore be considered to be of insignificant important in the problem relating to the origin of life.

S. Akabori in 1955 proposed a hypothesis concerning the origin of fore-proteins[19] He speculated that the first step is the formation of amino acetonitrile from formaldehyde ammonia and hydrogen cyanide. The second step is the polymerisation of aminoacetonitrile on a solid surface, probably in the state adsorbed on clay followed by hydrolysis of the polymer to polyglycine and ammonia and the third step is the introduction of the side chains into polyglycine by reaction with aldehydes or unsaturated hydrocarbons.

It has been shown that aminoacetonitrile can polymerise at quite low temperatures to give the polypeptide polyglycine[20] There is also other evidence to partially support the hypothesis[21] It is clear that quite complex polypeptides and proteins could be formed in this manner

It is probable that amino acetonitrile was formed when lightening (electric discharge) played up on the gases which were present in the primitive atmosphere of the Earth. From aminoacetonitrile as has been demonstrated by Akabori one can get very complex protein type of compounds under probable primitive Earth conditions

In the previous section I have described the results of experiments dealing with exposure of amino acids (singly or in combination with other amino acids with or without energy material with or without inorganic colloidal catalysts) to *sunlight to light from a thousand watt bulb or to ultraviolet light.* In all these experiments it was observed by us that in many cases a turbidity appeared in solutions and finally there was a deposite visible to the naked eye. Our first suspicion was that somehow some kind of infection has got into the cotton plugged flasks The experiments were repeated again and again taking all possible care to aviod contamination. In certain cases the deposits however continued to appear It then occurred to us that this deposit

might be a high molecular weight peptide containing several amino acids. The solutions were allowed to stand. In the case of tyrosine it was found that the browned solution gave gradually a brownish deposit. Hydrolysis of this deposit revealed the presence of several amino acids. Deposits in other flasks containing other amino acids were found to give qualitatively similar results. All this suggests that the formation of fore-proteins or proteinoids is a natural process Amino acids in aqueous medium can give rise to complex peptides and even fore proteins or proteinoids in presence of minerals with the help of sunlight. It is obvious that reactions in aqueous medium of the type suggested by S Akabori or by us are of far greater importance than the thermal pathways advocated by Fox and co-workers Here only natural conditions are used and the chances are that proteins with catalytic activity can be produced in this manner Dr M H Briggs informs me in a private communication that he used this technique on a mixture of 18 amino acids and was able to detect a weak estrase activity in the deposit. However the presence of weak estrase activity is not sufficient evidence for the natural combination of these amino acids. The result is however encouraging and I have no doubt in my mind that this is the right path for attempts to prepare proteinoids with possible enzymic activity

On Definition of Life

It is very difficult to define the term *life* N W Pirie has argued[?] that even with the existing organisms a rigid division between living and the non-living is not possible. He has aptly pointed out that we must recognise that life is not a definable quality but a statement of our attitude of mind towards a system [13] Keeping all this in mind we might say that the simplest definition of life or a living unit or system would be that it must show the qualities of growth division or multiplication and metabolic activity If all these characteristics are present in one system the system may be called living as it would acquire what Oparin has termed properties of a biological order This needs some explanation

Take the property of growth A crystal particle grows and retains its shape. A gel particle increases in size In the case of growth of a crystal from mother liquor there is increase in the material of the crystal but in the case of a gel particle the growth is merely due to hydration. The growth of a crystal is more like the growth in a living system but the growth of a gel particle does not even remotely resemble the similar phenomenon in the living systems

As example of division may be mentioned the breaking up of a [illegible] or a globule of mercury into fine particles This kind of division is different from the division in a living unit. In the former the initial mass is finely divided and is only getting divided into smaller units by mechanical means whereas in the latter the division results in the creation of similar smaller unit as a result of growth and budding

The metabolic activity of a living cell can easily be compared to any chemical change taking place in a unit. The essentials of metabolic activity are (i) selective assimilation of material from the environment and (ii) a system of selective chemical changes by which the unit can grow

The processes of growth division and metabolic activity can be observed to be taking place separately in a lifeless system A system can be said to be living only when all these take place in the same unit and that too in a systematic, harmonious and synchronised manner

Attempts have been made to prepare and study such type of particles which can show the phenomenon of growth division and selective assimilation. The nearest approach to this has been possible in the case of colloidal particles called coacervates.[131,132,133,134,135,136] It has been found that if solutions of biochemical polymers are maintained at suitable levels it aggregates form called coacervates They form spontaneously in mixtures of protein solutions with one or more other colloids and they appear to possess limiting membranes. They are of the size of small organisms and in hypertonic solutions they increase in size and eventually divide It has also been found that the absorption of the material into coacervates is highly selective. Thus they do exhibit properties closely resembling those of a living system and it has been suggested that the first organised cells originated in this manner[137] Academician Oparin also holds similar views.

I may here point out that the properties of some of the known coacervates do very much resemble those which are found in the living cell but there is a difference. In a living cell the phenomenon of growth, division and metabolic activity is found to be in the form of a systematic harmonious and synchronised behaviour This harmony and system is lacking in the known coacervates—even in those in which enzymes have been introduced artificially

Now we can define our attitude towards a system which we would call living

A system can be said to be living if it exhibits properties of growth, division and metabolic activity in a systematic harmonious and synchronised manner as is found in the living cell.

I am sure that most of the workers in the field dealing with the problem of the origin of life would subscribe to the above attitude towards a system which may be called living

Cell and its Origin

The cell of the present age is a very complicated affair It has nucleic acid for the transfer of heredity code and control on the formation of different protein molecules. It has adenosine phosphates for the liberation of energy for its metabolic and physiological activities. It has a number of enzymes

which coordinate their activities in a systematically controlled and harmonious manner Then there is the role of minerals. It has been observed[illegible] in [illegible] that mineral nutrients act as essential *negative catalysts* for the growth of the cell, and, the conditions for the optimum growth of the cell is a synonym for the condition for the maximum hydration of the protoplasmic material.[illegible] Thus we find that for formation of the present day cell a number of complicated organic substances are required as well as some minerals. There is a mineral abundance on the Earth and it had been so even in the prebiological era. The question of minerals in connection with the origin of the cell does not seem to present many difficulties. The production of the complicated organic compounds however present very challenging problems. A lot of work in different laboratories of the world is going on to study the *prebiological formation of these constituents* of the present day cell. Quite a measure of success has been achieved in certain cases.

For the origin of the cell there are two alternatives before us—

A. Nature first synthesised all the ingredients of the cell separately. It then brought them together in several stages. Finally when a suitable combination was evolved the first living cell was born.

B Nature might have followed some other way It might have practised on primitive much simpler cell like systems and finally evolved something which was *itself able to synthesise* the essential ingredients of the cell and ultimately became the cell itself.

First I would consider the hypothesis given under A. It is very difficult to imagine that first all the complicated ingredients of the living cell were synthesised by Nature separately Further merely on a consideration of probability it seems that their coming together suddenly to form one such the first cell is almost improbable. A mere *jumbling* up of the ingredients of the present day cell does not give rise to a cell. *The ingredients* must be arranged systematically and in a most specific pattern otherwise there would be no harmony To my mind this kind of arrangement is improbable. We are therefore left with the alternate hypothesis B. This hypothesis has been been very little explored Krishna Bahadur and S Ranganayaki have proposed a probable and cogent theory in support of this idea of the origin of the first animal cell [illegible]

In the opinion of Krishna Bahadur and S Ranganayaki matter has two more properties other than those which are well known and they helped in the process of emergence of life from non-life These two properties are

(i) *Under suitable conditions matter duplicates itself*

(ii) *A system of matter always tries to adjust to the milder changes in its environment*

Thus when there is a possibility of formation of innumerable molecules of different types and of the same thermodynamic level and needing same energy of activation, that molecule will be synthesised in larger number which is already in existence in the system.

According to them this rule is applicable to the formation of atoms, molecules or any bigger aggregates of molecules. Further

"If a mild change is introduced in a system of matter the system will so adjust itself that the effect of this change is counteracted giving rise to a slightly modified system of the matter The degree to which the system can adjust depends upon the particular system of matter in consideration and the amount of energy at the disposal of the system"

This type of adaptability has been observed in certain enzymic systems studied by Krishna Bahadur and co-workers. Gruwitsch[144] noticed the formation of tyrosine in a solution of glycine in presence of a trace of tyrosine.

Pette[145] has suggested the possibility of replication of protein molecules by a computer like mechanism. Bahadur and Ranganayaki believe that there is a way of making such a computer like mechanism inside a tiny globule of protein or protein like material and then the '*Computer*' would operate Since Krishna Bahadur and coworkers at the University of Allahabad and myself and H. D Pathak have succeeded in preparing units capable of growth, multiplication and metabolic activity in an organised manner it would be worthwhile to describe the theoretical ideas of Bahadur and Ranganayaki on which this achievement is possibly based These ideas would briefly be considered under the heads (a) source of energy (b) working mechanism of globule computer (c) globular wall, and (d) formation of units capable of growth division and metabolic activity

(a) *Source of energy* In the present day cell the metabolic activity is mostly controlled by adenosine phosphates which act as agents for energy transfer It is quite possible that the primitive cell might have had some other source for energy transfer Cells can be successfully grown in a medium where the only source of energy is the chemical reaction taking place in the medium.[146-147] Solar energy in the form of light can also be utilised by the organism if some such chemical was present in the medium which stored the solar energy during exposure and in the dark liberated it slowly The role of molybdenum in microbial fixation of nitrogen is well known. Molybdenum is also known to act as a catalyst in photochemical fixation of nitrogen.[148,149,150,151] It can well serve the purpose of adenosine phosphates in a primitive type of cell

(b) *Working mechanism of globule computer* "Suppose a globule is formed and it has such proteinous material that it does not solidify but retains its

viscous and plastic texture, and the globule has some source of energy to provide a rotatory motion to its contents, the flow of which starts from the centre goes to the circumference and returns again to the centre. Let us suppose this globule has a permeable wall. The materials present in the surrounding medium will enter into the globule passing through its permeable wall. If this wall has selective permeability it will allow the passage of some molecules and block the entry of a few others.

These molecules which enter the globule will come in contact with a layer of proteinous molecules which is being continuously changed because of the rotation of the inside material of the globule. Single molecular lines of peptide like compounds will come in contact with the incoming molecule and one after another and linkage by linkage of peptides between different amino acids, will pass through this contact. Thus all the molecules of the globule will in due course come in contact with this freshly entered molecule. If any of these globule substances has any catalytic activity with the incoming material, it will combine with it and will be dragged along with the globular material towards the centre of the globule. The incoming molecule thus caught will be reacted to whatever degree possible and will simultaneously continue its journey along with the reacting molecule or it may make the reacting species so heavy that they are left in the centre till the reaction is complete, and the reacting unit is free to continue its journey and new products of the incoming molecule are formed.

The products of the first reaction will come in contact with the other peptides which will be coming and leaving the centre and a series of reactions whichever is possible will continue till the products are either consumed up in building the material of the globule or there will be a stage when no further degradation of the residual product is possible and it will be discarded out of the globule.

"For the degradation products formed by the first reaction of the incoming material with the proteinous material of the globule, it is necessary that they remain in position where they can be further reacted by other molecular species of the globule Thus it is necessary that these degradation products of the incoming molecule should not go in solution and thus will be dispersed throughout the globule and decrease in concentration but should remain engulfed in the viscous material of the globule. Further degradation and synthesis will follow The proteinous matter of the globule will be continuously visiting these newly formed products during the course of the movement of the globular matter and different protein structures will be getting exposed to them and a series of reactions, if possible thermodynamically and with the energy of activation achievable will continue Thus the peptides of the globule even with their infinitely small enzymic or catalytic activity will be effective enough and there will come a stage when the material entering the globule will be ready to get converted

to some protein structure. At this stage there may be a theoretical possibility of forming a number of protein molecules with the material under consideration and with the energy available in the system but under these circumstances choice will go to the protein structures already present in the globule and more of these molecules will be formed as compared to other random protein structures. Thus the globule will grow in size.

"If the wall of the globule is flexible the newly formed material will remain inside the globule and it will become bigger in size. However soon will come a stage when the globule wall will not be able to hold any more of the material. At this stage the weak point of the wall will bulge out as a bud. Then the tension within the globule will decrease and it will again acquire a spherical active shape best suited for the whirling motion described within the globule Here the adaptability to the environment, described earlier as inherent property of matter has come in force. The huge size of the globule was unfavourable for the globule so it adjusted itself by giving out a bud thus acquiring a state of lesser tension.

The process described above would be repeatable in the bud. After sometime various stages of budding and clusters of globules with mother daughter and other globules derived from the original globule all attached together should be seen.

(c) *Globular wall* The globule described in (b) is surrounded by chemicals. In course of time these chemicals will modify it making its permeability more and more selective. It may be mentioned here that Mueller and coworkers[1 2] have prepared stable, bimolecular lipid and proteolipid membranes capable of self sealing and these membranes get modified by a variety of water soluble macromolecules which are spontaneously absorbed from the environmental solution. The wall may have such absorption property for water soluble macromolecules and at the same time it may also be permeable to a number of chemicals present in the solution. It is possible that this globule wall may become multilayered due to the precipitation of globular peptides like Liesegang rings [39 40] by the incoming chemicals of the medium, giving a still more complicated wall with more specific properties

(d) *Formation of units capable of growth division and metabolic activity* According to Bahadur and Ranganayaki the formation of the nucleus in the globule would be a natural process.

A few of the products of the incoming material will be retained in the globule preferably in the centre, where the currents of all the sides will converge and start. If the products are useful in the process of duplication or help in some way in adjusting the globule to the mild unfavourable conditions, there may remain in the central portion for sometime. Thus the centre will become a little more dense than the rest of the material of the globule and

the fluid motion of the globule which was first starting from the centre going to the circumference and converging again to the centre, will now start a little distance away from the central dense mass.

It is in this manner that the first dense central portion of the globule is formed. In due course of time it would evolve into a nucleus. By this time the globule would no longer remain an inactive globule but would become a unicellular unit capable of growth, division and metabolic activity in a systematic and harmonious manner. At this stage according to our definition we would be justified in calling it a living unit. These have been termed JEEVANU which in Sanskrit means particles of life.

Thus in short, the matter of producing units capable of growth division and metabolic activity is a question of putting the matter in such a way that it can manifest its properties and is like a surgical operation where the things are set right by necessary alterations and adjustment and the body does the rest. Once a globule of peptide is prepared with membrane, a source of energy is provided and necessary mineral and other nutrients are put in the medium and the system is allowed to stand free from bacterial infection the inherent properties of matter come in force and the activity which we call life comes into operation.

EXPERIMENTAL PRODUCTION OF UNITS CAPABLE OF GROWTH, MULTIPLICATION AND METABOLIC ACTIVITY

The experimental work described in this section is the joint work of Krishna Bahadur, H. C. Verma, R. B. Srivastava, K. M. L. Agrawal, R. S. Pandey, Indra Saxena, A. N. Malviya and Vinod Kumar of the Chemistry Department of the University of Allahabad, and, the author and H. D. Pathak of the Department of Chemistry, Th. D. S. B. Government College, Naini Tal.

These units can be prepared in several ways. Some of the important ways are described below.

1 *The following mixture was prepared in eight 250 ml pyrex conical flask.*

Paraformaldehyde	0 2 g
Molybdic acid	0·01 g
Ferric chloride	0 01 g
Water	100 ml

Flasks containing these mixtures were cotton plugged and sterilised at 15 lbs pressure for 30 minutes. The mouth of these flasks were covered by polythene sheets and four of these flasks were covered with four folds of [illegible] black cloth and four of the flasks were kept as such for exposure. At the

flasks were kept in sunlight for about 8 hours of exposure each day The temperature between this period of exposure remained between 15 to 25

After an exposure of 500 hours the mixture started becoming turbid. It was examined under microscope and globules of size 0 25 to 0 5 μ appeared in the mixture. Some of these had some mobility and appeared to have a dark central portion and an outer membrane. Similar mixtures kept in dark did not show the formation of such globules.

These globules were kept under observation during the exposure which was continued for 1000 hours. During this period it was noticed that the globules became bigger in size (1to1 5 μ) and showed various stages of budding After the said period of exposure the active units were separated by centrifuge and dried in a vacuum desiccator over anhydrous calcium chloride. The weight of the dried material obtained in the four flasks were 0 0326 g 0·0325 g, 0 0330 g and 0 0328 g The environmental medium was also dried The dried material of the globule and the environmental media were analysed for their free amino acid contents. The globular matter did not show the presence of any free amino acid but the environmental material showed the presence of glycine and alanine. These dried materials were hydrolysed with 1 ml of 6N hydrochloric acid and 5 ml of distilled water in sealed tube by heating in water bath for 20 hours The hydrolysed product thus obtained was filtered and dried in vacuum desiccator containing fused sodium hydroxide and anhydrous calcium chloride. The dried material thus obtained was treated with 2 ml of water and again dried in the desiccator Chromatographic analysis showed that dried material of the globular matter contained glycine, alanine, arginine, histidine, lysine aspartic acid and glutamic acid together with a few more faint spots which could not be positively identified. The hydrolysed product of the environmental medium did not show any spot other than glycine and alanine.

2 *The following mixture was prepared and exposed to sunlight after sterilisation at 15 lbs pressure for 30 minutes*

Citric acid	1 60 g
Ferric oxide sol.	40ml
Molybdenum oxide sol	40 ml
Distilled water	140 ml

As in previous case a similar mixture was covered with cloth and kept near the exposed mixtures.

A turbidity was observed in the mixture exposed to light after 20 hours' exposure time. This turbidity however disappeared giving a lear solution. No such change was observed in the mixture kept in dark. It remained clear and yellow in colour as was in the beginning The mixture exposed to light was observed to develop a pink colour after an exposure of 300 hours and started becoming turbid on further exposure. After an

exposure of 450 hours suspended particles were observed which settled at the bottom of the flask as a yellow mass leaving a clear yellow coloured solution. The number of these particles increased for a few days. The mixture was examined at this stage under oil immersion microscope at 1000 magnification

0 02 ml of the well shaken sample of this mixture exposed to light was taken out from the flask with a sterilised micropipette under aseptic conditions and a slide of it was mounted and studied under the microscope. Many needle shaped crystals varying in size were observed in all of the microscopic views A few clusters of these crystals were also observed. However a few round objects were also observed which had a glowing centre with a dark circular outline when viewed with 20X eyepiece (ca. ,000 magnification) These circular objects differed in size. To the periphery of a few such objects smallar circular objects were also observed to be attached and these appeared like budding of yeast cells. These circular objects were counted and their number recorded. Average count was 1·80

After 5 days another 0 02 ml of the well shaken mixture was mounted and viewed unde the microscope. It was found that the number of round objects as well as the needle shaped crystals have increased. Many round objects could be seen attached to the needle shaped crystals. The average count of these objects was now 8 66

After another five days it was observed that the number of circular objects has increased much and could not be counted as such. 1 ml of the thoroughly shaken mixture was taken out aseptically and was diluted to 10 ml in sterilised distilled water 0 02 ml of this mixture was mounted and viewed under the microscope Average count in terms of previous reading was 81·6 It was observed that two to three round objects were clinging to each other in clusters

After a lapse of a month again these round objects were observed under the microscope and their number was counted after diluting to 10 times. Average count in terms of previous readings was 117

3 *Formation of active units in aqueous solutions of amino acids on long exposure to light*

Experiments can be carried out with many amino acids but details of experiment with tyrosine are given here as some of the changes described can be followed even visually Chromatographically pure amino acids were used and double distilled water was employed in all experiments

(i)	L-Tyrosine	0.20 g
	Colloidal molybdenum oxide	40 ml
	Distilled water	500 ml

(ii)	L-Tyrosine	0·28 g
	Colloidal vanadium oxide	40 ml
	Distilled water	500 ml
(iii)	L-Tyrosine	0 28 g
	Colloidal ferric oxide	40 ml
	Distilled water	500 ml

All of these solutions were taken in quartz and pyrex flasks and sterilised for 30 minutes at 20 lbs pressure in an autoclave Exposure to sunlight was carried out day after day for at least 600 hours (in certain cases upto 1000 hours or more) In the case of exposure to ultraviolet light the solution in quartz flasks were exposed to light from a 300 watt high pressure mercury quartz lamp for 15 hours Though changes in all these solutions were observed when exposed to sunlight or ultraviolet light but no such changes were observable in the dark.

During the period of exposure as has been mentioned earlier these solutions turn brown Chromatographic analysis at different periods of exposure revealed the formation of other amino acids and peptides. The solutions were allowed to stand under aseptic conditions for some months. A brown sediment was found to be depositing in these solutions. This was separated by centrifuge, as described earlier and subjected to hydrolysis in the usual manner Chromatographic analysis of hydrolysate showed the presence of several amino acids Microscopic examination revealed the presence globules varying in size from 0.3μ to 1μ. It may be mentioned here that several type of objects are visible in the field of vision of the microscope There are mineral grains brown proteinous material, some crystals and coloured active globules. The active globules had well defined walls and the motion of the black spec inside was very much like the motion in a live cell. These particles grew in size and divided. They also formed conglomerates and frequently presented a picture resembling the typical behaviour of yeast cells. The colour of the active globules is different in the case of different metallic oxides. These active globules can be easily distinguished by their peculiar behaviour from the well known coacervates which are also present along with them.

4 *A case of quick preparation of active globules*

Several mixtures of the following composition were prepared.

Citric acid	0 04 g in 10 ml water
Molybdic acid	0 01 g in 10 ml water
Colloidal ferric chloride	10 ml
Mineral nutrient	10 ml
pH phosphate buffer	10 ml

These flasks were cotton plugged and the mixtures were sterilised in an autoclave at 15 lbs pressure for 30 minutes. The flasks were sealed with

polythene sheet and transparent cellotape. Four of these mixtures were exposed to sunlight as such and another four were covered with black cotton cloth and kept near the exposed mixtures.

No turbidity was observed in mixtures exposed to sunlight before 8 hours of exposure When this mixture exposed to light for 8 hours was kept in dark, a whitish turbidity appeared in it. Similar mixtures kept in dark from the beginning did not indicate such change

The turbidity was examined under oil immersion microscope. Small globules single and in groups of two and four were observed. The turbidity became denser and denser on exposure to sunlight and the clusters grew bigger in size. Huge colonies of 50 or more units were observed in a few days

Note Preparation of colloidal oxides and mineral nutrients mentioned in the above experiments

Ferric oxide sol used was prepared by the method described by Debray[163] and Krecke.[164] The concentration of ferric oxide in sol was 0·8 g/litre.

Molybdenum oxide colloid was prepared by the method of Graham[165] from potassium molybdate. Colloidal vanadium oxide was prepared by stirring ammonium vanadate (5 g) with dilute hydrochloric acid in a 200 ml pyrex beaker It was filtered washed and electrodialysed in parchment bag using distilled water

The buffer mentioned is ordinary sodium hydroxide-potassium phosphate buffer giving pH 6

The mineral solution used was prepared in the following manner—

Potassium sulphate	0.02 g
Sodium chloride	0 02 g
Calcium acetate	0 02 g
Magnesium sulphate	0 02 g
Zinc sulphate	0 002 g

The above was dissolved completely in 100 ml distilled water Then 0.7 [illegible] of potassium dihydrogen phosphate solution was added to it and the mixture was shaken till the whole thing went into solution This solution became turbid on boiling but is again clear on cooling

A possible correlation of organised structures found in carbonaceous chondrites [illegible] obtained by the conglomeration of active units of the type described in this [illegible]

In the section dealing with 'The Hypothesis of Extraterrestrial O[illegible] of life a mention was made of the microstructures observed in carbonaceous chondrites Numerous such structures have been found and a catalogued

such structures has been recently published (March 15 1963) as Technical Report No 32 398 of the Jet Propulsion Laboratory California Institute of Technology Pasadena California, U.S.A This report has been prepared by G Mamikunian and M. H Briggs In this section I want to consider the possible origin of the organised elements in some detail.

Claus and Nagy[19] have made the suggestion that the organised elements may be microfossils indigenous to the meteorites. Fitch and Anders [17,18] are tacitly suggesting that these organised elements are a variety of different structures, including silicate mineral grains opaque mineral particles, hydrocarbon globules, starch grains spores and other terrestrial contaminants. Fox[149] has suggested that they are organic polymers and sulphur in a super-cooled state-sulful droplets. Deflander[170] suggests that these are due to unspecified contaminants and artifacts According to Briggs[151] they are various mineral grains and aggregates of organic matter not necessarily of biological origin Claus et al have recently pointed out [72] that their petrographic studies indicate that the parent body of the carbonaceous chondrites was capable of supporting life. Mueller[173] has presented evidence indicating that Type V element is a rare limonite pseudomorph of troilite Thus it is clear that on the basis of the present available evidence it is not possible to arrive at any definite conclusion regarding the nature of these organised elements. However some very recent experiments conducted in my laboratory appear to throw a fresh light on the origin of these organised elements. These experiments are briefly described below

I have already described in the previous section that tyrosine solution containing colloidal oxides on exposure to light gives rise to several amino acids and peptides, and, ultimately to active type of globules or units capable of growth, multiplication and metabolic activity Further experiments were carried out with these solutions by adding to them the following —

A. To the original solution containing tyrosine and colloidal molybdenum oxide (I would refer to it as tyrosine solution) the following were added

(i) Tyrosine solution 10 ml
3% ammonium molybdate solution 0 4 ml
3% ascorbic acid solution (water) 0 4 ml
Mineral nutrient (as before) 1 ml

(ii) Composition same as in (i) except that in place 3% ammonium molybdate aqueous solution a 4 % ammonium vandate solution was used.

(iii) Composition same as in (i) but this time the ammonium molybdate solution was replaced by 5% aqueous solution of ferrous ammonium sulphate.

B To the solution containing tyrosine and colloidal vanadium oxide (referred to as tyrosine solution below) the following were added

(i) Same as given under A (i)

(ii) „ „ „ A (ii)

(iii) „ „ A (iii)

Thus this set of experiments differed from those given under A in charging the tyrosine solution containing molybdenum oxide with that containing initially colloidal vanadium oxide.

C. A similar set of experiments were designed as given in A (i), A(ii) and A(iii) The only difference was that in these experiments the starting solution was the old solution of tyrosine containing colloidal ferric oxide instead of molybdenum oxide.

Note The mineral nutrient was of the same composition as given in the previous section

All the solutions or mixtures described in A B or C were prepared under aseptic conditions and all precautions were taken to avoid any kind of outside contamination These solutions were exposed to sunlight and artificial light in cotton plugged flasks After very short exposures of a few hours very interesting changes were observable under the microscope An exposure of 8 hours of sunlight (2 days) or an exposure of about 30 hours to a 100 watt bulb is sufficient in most cases to bring about interesting changes.

In the old original tyrosine solution containing molybdenum oxide several types of structures can be seen. Some of the units show whirling motion. Single and small aggregates having bluish colour are observable. Generally most of the structures showed tendency to divide by budding. Generally single units were more prevalent than clusters. Some of the units and forms are given in Fig 4-8. The magnification in Figs. 5 and 6 is about 500, that in Figs. 4 and 7 about 1100 and in Fig 8 about 1300

When solution A (i) was used various types of structures could be observed under the microscope Some important type of structures were single active globular units (Fig 9) big globules with bright bluish colour grains (Figs. 11 13 14) aggregates of smaller units (Fig 10) bigger aggregates of various shapes (Figs. 12 15 16 17 18) Besides those in the Figs. mentioned above there were also several other types.

In case of A (ii) there were smaller units and bigger clusters were seen under the microscope. As compared to A (i) type the round globules and clusters were much fewer There was a tendency for the formation of filamentous microstructures as shown in Figs. 19 and 20 (magnification about 1000)

When A (iii) solution was used there were numerous bigger globules. Some of the interesting aggregates are shown in Figs 21 and 22 (magnification about 1000)

In old solution of tyrosine containing colloidal vanadium oxide micro-units of single double or in clusters were seen Single units were motile and showed a whirling motion. Fig 23 shows sufficient details to indicate the structure of these units The boundary line of these units was reddish and appeared to be composed of small granules. A peculiar cluster seen is shown in Fig 24 (magnification about 1000)

In case of solution B (i) globular and long aggregates were found in abundance Single units appeared to grow up to a certain extent only Fig 26 shows a linear type of aggregate and Fig 25 represents another type.

B (ii) solution showed a collection of various kind of structures. Even bigger globular type (Fig. 27) showed a tendency similar to budding (Fig 28) Fig 29 and 30 represent some peculiarly shaped aggregates. The linear structures were fewer in number in this solution

In solution B (iii) the types of aggregates found were many Some representative examples are given in Figs. 31 to 35 Some of structures such as given in Fig 33 were very big and extended much beyond the field of vision in the microscope Approximate magnification of these figures is about 1000

In the old solution of tyrosine containing colloidal ferric oxide were found several active units. Fig 37 shows one such unit and Fig 38 gives a view from probably a different angle Fig 36 shows numerous units and small clusters. All these were photographed under oil immersion using 100X objective and 10X eyepiece on 35 mm film. They were enlarged later to about three times the size.

Solution C (i) Several types of structures were found-smaller units (Figs. 39 and 41) and bigger clusters (Fig 40 Fig 42 and Fig 43) The structure shown in Fig 43 was very long and very complicated. There were several long ribbon like structures formed out of aggregates of single or complex units.

Solution C(ii) This mixture showed various insect like microstructures. These are shown in Fig 44-46 In this solution it appeared that the structures were more organised. There was however no definite general pattern traceable.

Solution C(iii) Very thick structures were seen Some representative examples are given in Figs 47-49

In all the cases mentioned above not only the structures referred to were seen but there were also crystals brown matter and other material. The structures shown in various figures (4 to 49) were composed of tiny coloured globules. The colours were different in different cases Some were black, some greenish and others brownish. Along with complicated aggregates single units were also observable.

Next a complicated mixture of amino acids was used. In 500 ml of double distilled water were put glycine (0 036 g) cystine (0.12 g) alanine (0 126 g) valine (0 118 g) methionine (0 016 g) leucine (0.246 g), iso-leucine (0 052 g) tyrosine (0.102 g) phenylalanine (0 132 g) tryptophane (0 0117 g) lysine.HCl (0.256 g) histidine (0 08 g) arginine (0.118 g) ornithine (0 02 g) proline (0 096 g) glutamic acid (0.5 g), aspartic acid (0 3 g) threonine (0 05 g) and serine (0 084 g) The mixture was sterilised and exposed to light After an exposure of 30 hours to sunlight in quartz vessels the solution acquired a brown tinge and eventually on further exposure, there was a brown deposit. If the mixture of amino acids contained sucrose also as energy material then again a similar brown deposit was obtained

10 ml of this solution was taken (with or without sucrose in the original solution) and to this was added mineral nutrient solution (1 ml) ascorbic acid (3% 2 ml) and ammonium molybdate (3% aqueous solution 0.4 ml) and double distilled water (6 6 ml) All this mixing was done under sterilised conditions and the solutions were finally exposed to light in pyrex vessels.

After an exposure of a few hours (10-30) several active units were seen in the solution when it was examined under a microscope using oil immersion and 10X eyepiece. As exposure time is increased they multiply rapidly and very complicated aggregates are formed. When sugar has not been added initially the type of structures found are shown in Figs. 50-55 and when the solution contained sugar also the type of structures found are shown in Figs 56-61 The structure shown in Fig 51 was a big globule with a shining inside. In Fig 56 is shown part of a complicated globular structure. The structure was bigger than the field of the microscope when objective (100X) All these structures are shown at about 1000 magnification in Figs. 50-61

Several of the aggregates described here have a remarkable resemblance with the organised structures found in meteorites It has been pointed out earlier that several amino acids have been found in carbonaceous chondrites. It appears very probable that some of the organised elements were also created in the manner in which aggregates of active units were obtained by us in the laboratory About half-a-dozen of the microphotographs of these aggregates were sent to Dr Briggs. He is of the opinion that these look remarkably like the type of structures found in carbonaceous chondrites

If the suggestion of Claus and Nagy about the extra terrestrial origin of these structures is valid then there is no doubt that processes of the type described in this paper might be taking place there Alternately if these structures in carbonaceous chondrites are due to terrestrial contamination, then too the method described in this paper points to a solution of the riddle. It is however yet too early to say anything very decisively in this matter

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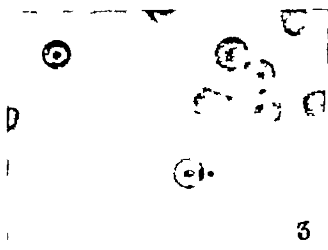


Fig 3 Microphotograph of th aeth units showing two units with small buds (magnification bout 1000)

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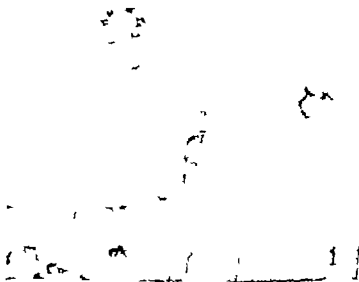
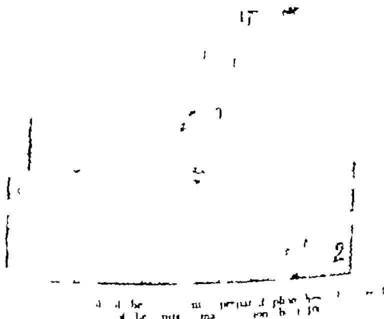
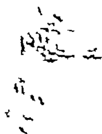


Fig 1 Microphotograph of the acti unit prepared photochemically small bud still attached to the parent unit (magnification fout [



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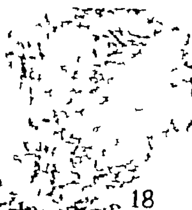




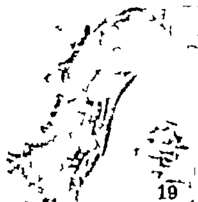
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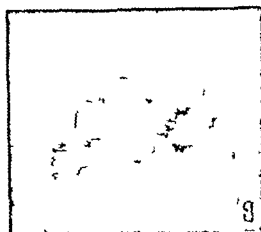
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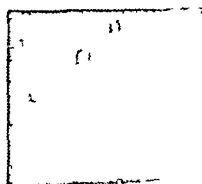


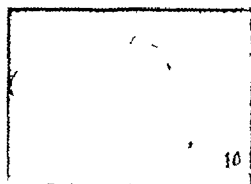
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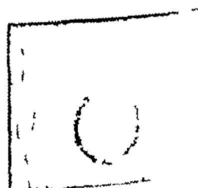


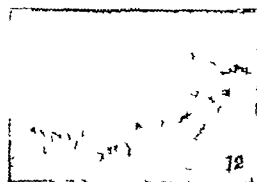
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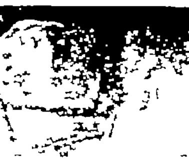

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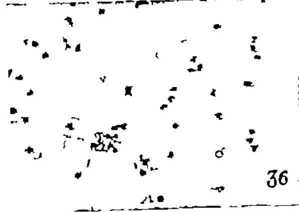



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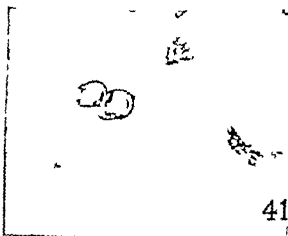
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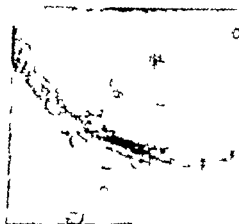


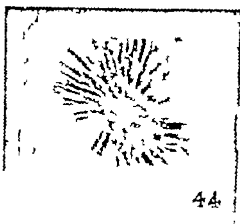

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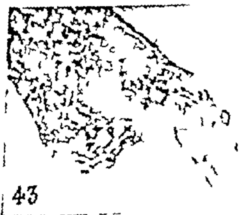


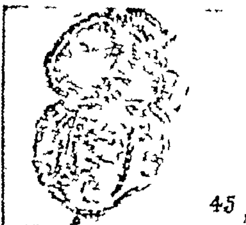

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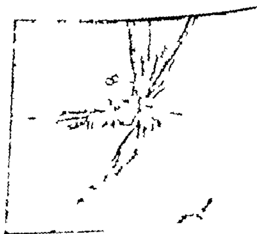

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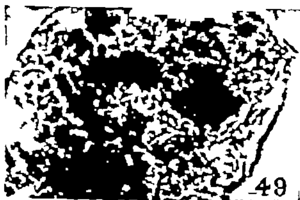

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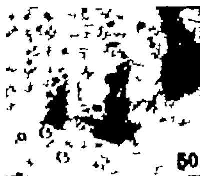

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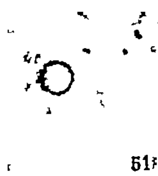



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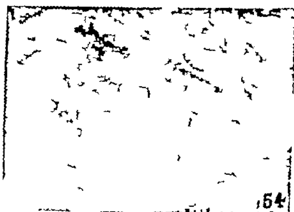

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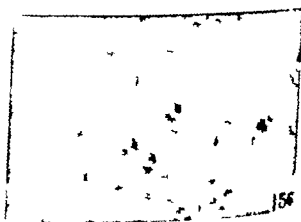

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VOLUMETRIC ESTIMATION OF IRON IN PRESENCE OF HCl by $KMnO_4$

TARA CHANDRA GUPTA AND ABANI K. BHATTACHARYA
Chemistry Department, Agra College Agra.

It is a well known fact that the volumetric estimation of ferrous salts can be most suitably carried out with a standard solution of $KMnO_4$ in the medium acidified with H_2SO_4, but the volumetric estimation in the HCl medium are not generally reliable These estimations in the presence of HCl are very much influenced by the concentration of the HCl. In order to conduct the preliminary study on the estimation of ferrous with $KMnO_4$, it was deemed necessary to obtain critically the limiting concentration of HCl which makes the titration permissible within an experimental error of $\pm$ 0·5%.

A. Skrabal[1] reported that manganaous sulphate added in sufficient quantity controls the effect of the moderate amount of HCl. George J Hough[2] observed that ferrous salts can be accurately estimated with a standard solution of $KMnO_4$ if H_3PO_4 alone was added previously to the solution. The use of a mixture of $MnSO_4$ H_2SO_4 and H_3PO_4 was recommended by Reinhardt—Zimmermann[3] and it was confirmed by later workers also.

In view of the foregoing observations volumetric estimations of ferrous ammonium sulphate have been carried out in the presence of HCl alone and in the presence of manganaous sulphate, phosphoric acid and Reinhardt—Zimmermann reagent. The limiting accuracy of these estimations have been shown and discussed in this paper

EXPERIMENTAL

Standard solutions of HCl, H_2SO_4, $KMnO_4$ $MnSO_4$ H_2O and $FeSO_4$ $(NH_4)_2$ $SO_4.6H_2O$ were prepared by using their A. R. quality Reinhardt—Zimmermann solution was also prepared (Jour Amer Chem. Soc., 1914 36 1429)

Observations

Table 1

Solutions used N/10.08 $FeSO_4(NH_4)_2SO_4.6H_2O$ N/10 $KMnO_4$
25 c.c. of $FeSO_4(NH_4)_2SO_4\ 6H_2O \equiv 24.8$ c.c. of $KMnO_4$
in the H_2SO_4 medium

Volume of $FeSO_4(NH_4)_2SO_4\ 6H_2O$ + $2NH_2SO_4$ in c. c.	pH	$KMnO_4$ required in c. c.
25+4	85	24 8
25+6	75	24 8
25+8	65	24 8
25+10	60	24 8
25+12	55	24 8
25+13	50	24 8
25+13 6	50	24 8
25+13 8	50	24 8

Table 2

Solutions used N/10.002 $FeSO_4(NH_4)_2SO_4\ 6H_2O$ N/10.023 $KMnO_4$
25 c.c. of $FeSO_4(NH_4)_2SO_4.6H_2O \equiv 25.03$ c.c. of $KMnO_4$
in the H_2SO_4 medium

Volume of $FeSO_4(NH_4)_2SO_4\ 6H_2O$ + 2N HCl in c c	pH	$KMnO_4$ required in c c
25+4	1 0	25 03
25+6	9	25 0[illegible]
25+8	8	25 10
25+10	75	[illegible]5 10
25+12	70	[illegible]5 15
25+14	65	[illegible]

TABLE 5

25 c.c. of N/10 $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O \equiv 25$ c.c. of N/10 $KMnO_4$ in the H_2SO_4 medium.

Volume of Ferrous ammonium sulphate in c.c.	Concentrated HCl in c. c.	Manganese Sulphate in c. c	Syrupy H_3PO_4 in c c.	Reinhardt Zimmermann reagent in c. c.	$KMnO_4$ required in c c
25	1	0	0	0	25 1
25	2	0	0	0	25 1
25	3	0	0	0	25 1
25	4	0	0	0	25 15
25	5	0	0	0	25 15
25	10	0	0	0	25 30
25	3	25	0	0	25 05
25	5	25	0	0	25 05
25	10	25	0	0	25 5
25	3	0	3 5	0	25 00
25	5	0	3 5	0	5 05
25	10	0	3 5	0	25 20
25	10	0	8 5	0	25 20
25	3	0	0	25	25 00
25	5	0	0	25	25 05
25	10	0	0	25	25 20
25	15	0	0	25	25 20

DISCUSSION

It will be seen in Table 2 how the variations in the titre values of potassium permanganate take place by gradually increasing the concentration of HCl (2N). The actual value for 25 c.c of the standard ferrous ammonium sulphate of the prepared strength was equivalent to 25·05 c c

of $KMnO_4$. It will be interesting to note that the results of titrations varied only upto 0·2 % in a solution of within the range of pH 1·0 0·72, but when HCl was further added the percentage error increased upto 0.6% the pH being equal to 0·65. In the presence of H_2SO_4, however the titre value of $KMnO_4$ remained constant (24·8 c.c.) vide Table 1 within a much wider range of pH 0·85 0·3. The reason for the variations in the point of equivalence in the ferrous—permanganate titrations in the presence of HCl may be ascribed to the variations in the Eo values when the concentration of HCl is higher than the optimum value. It is supported by our observation (J.I.C.S. 1959 36 399)

On critically observing the figures given in Table 3 it will be seen that by adding concentrated HCl (specific gravity 1·18) in increasing amounts the percentage error in ferrous-permanganate titrations increases from 0·4 to 1·2%. But the error is appreciably controlled upto 5 c.c. of concentrated HCl (specific gravity 1·18) added to 25 c.c. of ferrous ammonium sulphate solution. Beyond 5 c.c. of concentrated HCl the percentage error increases appreciably inspite of the addition of manganous sulphate. Similar effect is also shown by syrupy phosphoric acid. The effect of syrupy phosphoric acid used as a preventive appears to be slightly greater in controlling the error than manganous sulphate. By adding both manganous sulphate phosphoric acid and sulphuric acid (Reinhardt—Zimmermann reagent) the preventive effect remains practically the same as is observed by adding syrupy phosphoric acid alone (vide Table 3). Similar preventive effect of H_3PO_4 in ferrous-permanganate titrations was observed by George J. Hough[2]

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STUDIES ON PLEUROPNEUMONIA LIKE ORGANISMS AND OTHER FILTERABLE AGENTS ASSOCIATED WITH CHRONIC RESPIRATORY DISEASE OF POULTRY

SURAJ BHAN SINGH
Department of Pathology & Bacteriology
U P College of Veterinary Science & Animal Husbandry Mathura

The present study was undertaken to determine the incidence of mycoplasma infection in poultry in various parts of the country to characterize the PPLO strains on the basis of cultural and biochemical tests and pathogenicity trials, to evaluate various antibiotics against PPLO by invitro sensitivity tests to measure the effectiveness of dipping of hatching eggs in antibiotic solutions for the control of egg borne mycoplasma infection and to search for some known or unknown filterable agent that may be present in association with Pleuropneumonia like organisms in cases of Chronic Respiratory Disease.

Taylor and Fabricants liquid carbohydrate medium with some modifications for isolation and Avian PPLO Diagnostic Antigen (Connecticut) for serology were utilized with considerable success.

Materials for isolation and serology included 1502 throat swabs and 1517 serum samples from birds from seven States viz, Madras, Mysore Orissa Bihar Andhra Pradesh, Madhya Pradesh and Uttar Pradesh. Ninety per cent of the 189 serum samples from the first five States were positive for PPLO agglutinins. Mycoplasma infection appeared to be of country wide prevalence. PPLO could be isolated from serologically positive as well as negative birds The overall picture of isolation and serology was 48 and 56 per cent respectively This indicated a fairly good deal of correlation between the two diagnostic procedures. The correlation of isolation or isolation and serology with lymphofollicular reaction in the lungs and/or trachea in grossly positive and negative cases was low Serological examination is considered useful to detect inapparent infection.

Very low incidence of infection was noted in desi birds. Ducks were also negative. The poultry farm maintained at the Ingraham Institute, Ghaziabad U P and the experimental flock raised at Pathology and Bacteriology Department, Veterinary College, Mathura were found to be PPLO free on repeated attempts

Out of 793 strains isolated, 191 were studied as regards their colony characters, rate of growth, fermentation of sugars, tetrazolium reduction and

haemagglutination None of the strains fermented mannitol Two major classes were distinguished one producing pathogenic type (small without nipple) and the other nonpathogenic type (large with central nipple) colony morphology Majority of the strains were nonpathogenic and nonhaemagglutinating Strains with almost all combinations of above characteristics were encountered It appeared that morphological, cultural, biochemical and serological characters taken together would be adequate to differentiate pathogenic from nonpathogenic PPLOS

Experimental trials were conducted in PPLO free chickens. The PPLO strains incited a significant positive antibody response by the combined intravenous air-sac route but no symptoms or lesions developed. Eye inoculations also failed in producing any pathological changes. The studies indicate that PPLO probably lose their pathogenicity on repeated subcultures in artificial media.

Invitro sensitivity trials showed that the six antibiotics tested against two pathogenic PPLO strains were in the following order of diminishing activity tylosin DOL, erythromycin streptomycin dihydrostreptomycin and penicillin.

Seventy six PPLO strains were isolated from 330 composite samples of 1563 eggs. The frequencies of isolations were 12% 15% 26% and 35% of the samples of unfertile, weak or dead germs dead in shell and pipped eggs respectively indicating a high incidence of egg-transmission

Eggs from CRD infected flock were prewarmed and dipped for half an hour in 1600 ppm tylosin or 2000 ppm erythromycin solution at 5°C. One hundred and eighty chicks from the former and 132 chicks from the latter treatment were obtained Forty chicks examined at two weeks of age from tylosin lot were serologically negative. No isolations could be made from 43 dipped unfertile or dead in shell embryos from both antibiotic groups. Thirty two necropsied chicks similarly did not yield any PPLO. The application of such a procedure in raising PPLO free flocks appears to be of great promise

A virus like agent was isolated from cases of respiratory infection. This entity constantly produced pock-like lesions on the C. A. membrane and was non-haemagglutinating The implications of the occurrence of such agents alongwith PPLO infection are discussed

The importance of carrying out further research work to determine the criteria of Pathogenic PPLO strains including experimental transmission, the association of other filterable agents and also the necessity of raising PPLO free flocks by antibiotic dip treatment is stressed

DETERMINATION OF OPTIMUM pH FOR THE ACTIVITY OF CAECAL PROTEASES OF ORCHESTIA GAMMARELLA PALLAS

V P Agrawal
D A V College Muzaffarnagar

Introduction

The animal, *Orchestia gammarella* Pallas is very commonly found at high waters under weeds and stones sometimes several miles away from the sea They feed on any vegetable matter they come across The author has been able to feed them even on small pieces of filter paper soaked in sea water They eat very great amount of food daily probably they eat indiscriminately whatever comes their way if it is only not too dry and not too hard

The alimentary canal of *Orchestia* consists of the foregut the midgut and the hindgut. The foregut includes the mouth oesophagus and stomach the latter is further divisible into an anterior cardiac and posterior pyloric chamber The cardiac stomach serves for the mastication of food while the pyloric stomach acts as the filter apparatus. From the floor of the midgut, at its junction with the stomach, arise two lateral pouches which extend backwards and soon divide into two pairs of ventral hepatopancreatic caeca These caeca serve both for the secretion of the digestive enzymes and for storing the food in the form of fat globules

Here in this present work the optimum pH for the activity of the proteases secreted by the caecal cells of *Orchestia* has been determined.

Material and Methods

A large number of specimens of *Orchestia* were collected from Whitstable coast where they occur in abundance (Newell, 1954)

In order to prepare the extract of the ventral caeca about 200 animals were dissected and the caeca were taken out which were ground up with a little thymol and a drop of glycerol until a fine uniform emulsion was obtained. This was, then, diluted to about 10 per cent with 50 per cent glycerine the rest of the tube was filled with toluene The extract was kept at room temperature for about 48 hours before it was used for experimentation.

Determination of Optimum pH

To investigate the optimum pH for the activity of proteases the rate of liquification of gelatine was observed over hourly intervals with the enzyme, acting in media ranging in pH from 4 1 to 9 1

One ml of 10 per cent gelatine solutions were placed in different tubes and 1 ml of alkali or acid of different normalities were added in order to get desired pH. These substrates were then incubated with 1 ml of caecal extract at room temperature. The pH in these different tubes was noted before and after the experiment which did not show any appreciable difference. The following figures were used to describe the degree of liquification 0- completely solid 1- solid but small pieces may be torn off by shaking solid but surface moves somewhat when the tube is shaken 3- soft 4- half liquid 5- almost liquid 6- entirely liquid.

The following table shows the degree of liquification at different intervals at known pH

TABLE

Interval in hours with degree of Liquification

S.No	pH	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	7 hrs
1	4 1	0	0	0	0	0	0	1
2	5 0	0	0	0	1	1 2	1 2	1–
3	5 8	0	1	1	1 2	1 2	1–2	3
4	6 1	0	1	1	1 2	1 2	2 3	3–4
5	6 5	0	1	1	1 2	1–2	2–3	3–4
6	7 1	0	1	2	3	4	5	5–6
7	7 5	0	1	2	3	4	5	3–4
8	7 7	0	1	2	3–4	5–6	5–6	6
9	8 3	0	1	2–3	4	5–6	6	6
10	8 7	0	0	1	1 2	4	5	5–6
11	9 1	0	0	0	1	1–2	2	3–3

The appended graph (Text Fig 1) and the above table, shows that the optimum pH for the activity of proteases is on the alkaline side and lies between 8 and 8 5

SUMMARY AND DISCUSSION

Orchestia gammarella very commonly found at high waters feeds on any matter if it is only not too dry and not too hard.

The ventral hepatopancreas of *Orchestia* as the name signifies acts both like the liver and pancreas of the chordates i.e it serves to store the food material and also secretes the digestive enzymes Nicholls (1931) also observed

that the caecal cells of *Ligia* are secretory in nature. Patrick (1926) studied the nature of the caecal cells of *Ligia* and found that the larger cells of the caeca store the reserve food in the form of fat globules. Similar observations have been made by the author with respect to the caecal cells of *Orchestia*.

It has also been observed, as by Yonge (1924) in *Nephrops* that the caecal proteases of *Orchestia* act optimally in the alkaline medium i. e. between pH 8 and 8.5

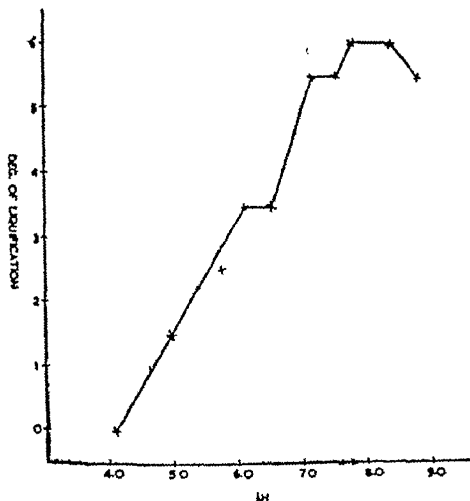


Fig. 1

Graphical representation of the activity of the caecal proteases of *Orchestia gammarella*, at different pH, on the liquification of gelatine

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BEHAVIOUR OF HYDROGEN PEROXIDE TOWARDS SOIL ORGANIC MATTER

A. N. PATHAK AND V. K. DIXIT
Agricultural Chemistry Section, Govt. Agricultural College, Kanpur

Organic matter is known to contribute to a great extent to several physical and chemical properties of soils which are of greatest significance in crop production. The decomposition of organic matter has been studied in greater detail covering various aspects, but comparatively little work has been done on the oxidation of organic matter in soils. Robinson[18] has suggested that the accurate mechanical analysis of soils is possible only after the organic matter present in the soils has been destroyed. Hosking[9] has shown the oxidation of soil organic matter to be a function of the H-ion concentration of the soils and suggested that the proportion of organic matter oxidised is a definite function of the clay content of the soil. Kerr[11] reported a variation of 7% to 28% in the absorptive capacity of soils due to organic matter. Bartlett *et al.*[2] while studying the effect of hydrogen peroxide on the exchange capacity of Maryland soils observed that the fine textured soils showed the greatest loss in exchange capacity by oxidation. Drosdoff and Mills[7] have shown that the H_2O_2 treatment of soils containing hydrated type of weathered mica resulted in an increase in the coarser separates and a consequent decrease in the finer fractions while in the case of soils containing weathered mica not of hydrated type the opposite effect was observed. Burr and Russell[6] observed that the removal of organic matter from soils resulted in the lowering of scouring point decreasing the plastic range and increasing the toughness and solidity. Russell and Wehr have suggested that the organic matter affects the plasticity of soils. Baver[4] reported that the oxidation of organic matter produced a marked lowering of both the upper and lower plastic limits on the moisture scale. Rich *et al.*[16] have observed a reduction of 46·1% in the cation exchange capacity of soils as a result of H_2O_2 treatment. Recently Savage *et al.*[19] found that the intermediate products formed by H_2O_2 oxidation of humic acids contained N_2 as part of their molecular structure.

EXPERIMENTAL TECHNIQUES

Six soil samples with varying organic matter content were air dried and crushed to pass through 2 m.m. sieve. Usually 100 gms. of each soil was oxidised by the desired concentration of H_2O_2. The soil was stirred from time to time and the oxidation was taken to be complete when there was no frothing. After complete oxidation the soils were filtered with suction on a Buchner funnel and thoroughly washed with distilled water. The soils were then air dried and crushed to pass through a 2 m.m. sieve. The soils thus prepared were used for these studies.

In order to study the effect of cations on the oxidation of soil organic matter three of these soils, rich in organic matter were converted into Ca, H and Na soils by leaching them by Normal solution of their chlorides except in the case of H-soil. The soil saturated with H ions was prepared by leaching the soil with N/20 HCl. After saturation the excess of HCl was removed by washing with 60% alcohol. The soils were then air dried and crushed to pass through 2 m.m. sieve. The following determinations were made —

(a) Organic Carbon by Walkley and Black[19] method.

(b) Total Nitrogen by Kjeldahl's method as modified by Bal[1]

(c) Cation Exchange capacity by Ammonium acetate method as described by Piper [14]

Results and Discussions

TABLE 1a

Effect of Hydrogen peroxide on C & N of different soils

Sample No	Original			Residual			Loss	
	C%	N%	C/N	C%	N%	C/N	C%	N%
1	2 434	1440	16 9	0 558	036	15.5	77 07	[illegible]
2	2 126	1320	16 1	636	0436	14 5	70 [illegible]	[illegible]
3	1 468	098	14 9	464	0382	12 1	[illegible]	[illegible]
4	1 116	084	13 2	415	0352	11 7	62.7	[illegible]
5	0 550	048	11 4	240	023	10 4	56 3	[illegible]
6	0 490	045	10 9	.264	027	9 7	46 3	[illegible]

TABLE 1b

Effect of different amounts of 7.5% H_2O_2 on C & N of soil No. 1

[illegible] 7.5% H_2O_2	Residual			Oxidised	
	C%	N%	C/N	C	N
50 c	535	036	15 4	78 01	[illegible]
100	462	034	13 6	81 01	[illegible]
150 c	385	034	10 4	84 1	[illegible]
200 c c	268	030	8 9	[illegible] 9	[illegible]
250 c c	.210	028	7 5	91 3	[illegible]
300 c	200	026	7 4	91 7	[illegible]

TABLE 1c

Effect of different concentration of H_2O_2 on soils No 1 & 2

Concn of H_2O_2	Residual			Oxidised	
	C%	N%	C/N	C%	N%
Sample No. 1					
15%	268	030	8 9	88 9	79 1
7 5%	558	036	15 5	77 07	75
3 0%	764	046	16 6	68 1	68 05
Sample No 2					
15%	410	041	10 0	80 7	69 0
7 5%	636	0456	14 5	70 08	66 9
3 0%	852	0190	17 3	59 9	62 8

TABLE 1d

Effect of no of treatments on soils no 1 & 2

Sample No.	N of treatments	Residual			Oxidised	
		C%	N%	C/N	C%	N%
I	1	636	0436	14 5	70 08	66 9
	2	824	045	12 5	73 3	68 1
II	1	464	0362	12 1	68 3	61 02
	2	388	0360	10 7	73 5	63 2

TABLE 1c

Alkali Soils

pH	Original		Residual		Loss	
	C%	N%	C%	N%	C%	N%
9.2	6842	0422	5326	0400	22 15	5 2
8 8	4886	0392	4025	0380	17 66	3 06
The soils were acidified by N/10 HCl and then subjected to oxidation						
5 2	6842	0420	2024	0244	70 41	42 1
5 6	4886	0392	1932	0226	60 45	44 8

1 *Effect on Carbon & Nitrogen*

From the results given in table 1 (a) it is observed that as much as 77% of organic carbon in the soils studied is lost by H_2O_2 treatment. The loss in N% was found to vary from 40 to 75%.

Attempts were then made to study the effect of varying amounts of 7.5% H_2O_2 on the oxidation of organic matter (Table 1b). The oxidation was found to proceed with the additional amount of H_2O_2 until a point was reached near about 250 c. c. when more than 91% of the Carbon was removed and thereafter practically no further oxidation was observed. No significant change in N% was also observed as is seen from the narrowing down of C/N ratios. The soils were then subjected to second treatment of H_2O_2 and it was observed (Table 1 c) that the repeated treatment of the soil by H_2O_2 was more effective than the single treatment in removing C and N from the soils.

On changing the concentration of H_2O_2 (Table 1c) it was observed that the amount of *residual* carbon increases with the decrease in the concentration of H_2O_2 but the N% of the soils was not affected to the same extent as can be concluded from the C/N ratio which increased with the decrease in H_2O_2 concentration. The C/N ratio of the residual organic matter was found to increase with decreasing concentration of H_2O_2.

For the purpose of studying the behaviour of H_2O_2 towards soil organic matter present in alkali soils two alkali soils were treated with 7.5 H_2O_2. In the case of these soils a low order of oxidation (17% to 22%) was observed (Table 1c) but when these soils were acidified by N/10 HCl and there after treated with H_2O_2 the percentage of oxidation was found to increase considerably. This is further supported by the findings of Hosking[1] [illegible] pointed out that in alkaline solution a catalytic decomposition of H_2O_2 takes place in the presence of MnO_2 with Mn $(OH)_2$ as the probable intermediate compound.

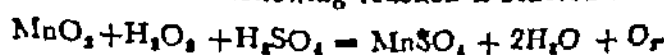


$$MnO_2 + H_2O_2 = Mn(OH)_2 + O_2$$



$$Mn(OH)_2 + H_2O_2 = MnO_2.H_2O + H_2O$$

In acid solution the following reaction is believed to take place



$$MnO_2 + H_2O_2 + H_2SO_4 = MnSO_4 + 2H_2O + O_2$$

On the basis of these facts it is quite obvious that the oxidation of organic matter in alkali soils will be low as compared to normal soils

TABLE 2

Cation Exchange capacities of the soils as affected by H_2O_2 Treatment

Sample No	Cation Exchange capacity (m %)	C E Capacity (m %)			
		5% H_2O_2	7 5% H_2O_2		15% H_2O_2
			Ist treatment	II treatment	
1	24 88	17 42	15 45	15 00	14 68
2	22 46	16 54	14 64	14 24	14 00
3	18 00	14 50	13 36	13 00	12 48
4	15 42	11 84	10 24	10 01	9 86
5	11 40	9 00	8 24		7 80
6	10 80	8 86	8 00	—	7 90

2 *Effect on Cation Exchange Capacity*

The cation exchange capacity of the soils was found to decrease due to treatment by H_2O (Table 2) This is in accordance with the findings of Kerr[9] Bartlett *et al.*[3] and Baver [4] On varying the concentration of H_2O_2, it was observed that the reduction in cation exchange capacity was directly proportional to the concentration of H_2O_2 used The second treatment of soils by H_2O_2 resulted in further reduction of cation exchange capacity

A linear relationship between cation exchange capacity and organic carbon content of soils was observed (Fig 1) but there was no such relationship between nitrogen content and cation exchange capacity of the soil The reduction in cation exchange capacity due to oxidation of organic carbon conforms the findings of Hosking and Piper Hosking[10] and Pathak, Mukherjee and Shrikhande [14]

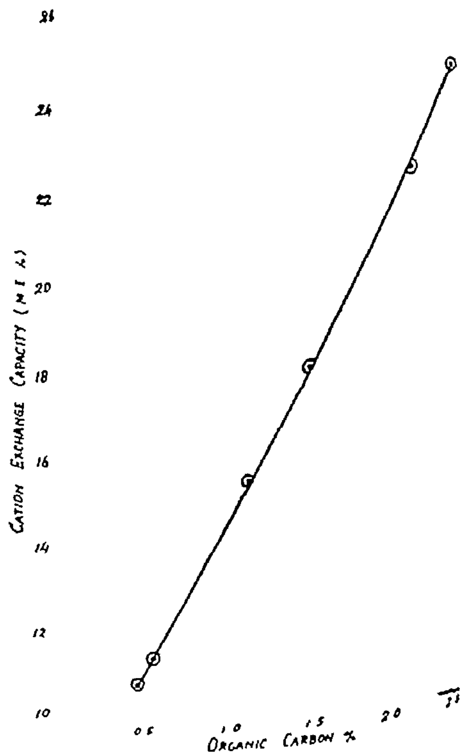

24
22
20
18
16
14
12
10
CATION EXCHANGE CAPACITY
0.5
1.0
1.5
2.0
ORGANIC CARBON %

TABLE 3

Effect of H_2O_2 on soils saturated with different cations

Different Cations	Sample No. 1			Sample No. 2			Sample No. 3		
	Original C%	Residual C%	% Loss	Original C%	Residual C%	% Loss	Original C%	Residual C%	% Loss
H	2 434	0 594	75 5	2 126	0 600	71 7	1 468	0 438	70 1
Na	2 434	1 296	46 7	2 126	1 266	40 4	1 468	0 912	37 8
Ca	2 434	1 448	40 5	2 126	1 358	36 1	1 468	1 012	31 06

3 *Effect of Exchangeable Cations*

The effectiveness of H_2O_2 in oxidising organic matter of the soil decreu sed with the type of the cations saturating the soil H>Na> Ca This may be explained by Mattson's[12] study on the electrical m gration of soil colloids saturated with different cations, that the charge is the highest in Na-saturated particles which indicates that this ion dissociates more extensively from the surface of the particles as compared to Ca saturated particles It means that the slow detachment of Ca will inhibit the oxidation process and so the oxidation will be lower in case of Ca soils. This may further be supported by the findings of Wiegner and Pulmann[20] who observed that the displacement of H+ by NaCl led to a weaker dispersion acidity while the Ca permutite produced an alkaline dispersion effect A more or less similar situation arises in the case of Ca-soils causing lower oxidation.

TABLE 4

Effect of H_2O_2 on raw organic matter

Raw Organic matter	Original C%	Carbon %		
		1st Treatme t	2nd Treatment	Reaction catalysed by the soil
Sanai	56 80	45 64	38 46	22 18
Moong T_1	54 64	42 80	35 54	20 44
Wheat Straw	53 50	40 48	32 64	18 32
Paddy Straw	48 80	39 76	30 44	15 66

4 *Effect on Raw Organic matter*

Raw organic matter like Sanai, Moong T[1] Wheat straw and Paddy straw were treated by 7 ɔ/o H_2O_2 and decrease in the percentage of carbon was observed in all the cases (Table IV) The second treatment by H_2O_2 caused a further reduction in the percentage of carbon. After this a small quantity of soil was added to catalyse the reaction and it was quite interesting to note that the carbon percentage was reduced considerably due to the treatment. From thus it may be inferred that the raw organic matter is not easily decomposed by H_2O_2

TABLE 5

Ammonium Fixation as affected by H_2O_2 Treatment

Sample No.	Original Soil			H_2O_2 Treated Soil			
	NH_4—N added m.e. %	NH_4—N Leached m.e. %	NH_4—N fixed m. %	NH_4—N Leached m.e. %	NH_4—N fixed m.e %	Decrease in NH_4 fixation after H_2O_2 treatment m.e. %	% Decrease in NH_4 fixation
1	3 0	0 95	2 05	1 53	1 47	0.58	[illegible]
2	3 0	1 14	1 86	1 58	0 42	0 44	[illegible]
3	3 0	1 60	1 40	1 90	1 10	[illegible] 30	[illegible]
4	3 0	2 0	1 00	2 12	0.88	0 12	–

5 *Effect on NH_4^+ fixation*

In order to study the role played by the soil organic matter in NH_4 fixation an attempt was made to determine the amount of NH_4^+ fixed by [illegible] before and after oxidation of soil organic matter by two successive treatments of 15% H_2O_2

From the results given in table 5 it is observed that the removal of organic matter leads to decrease in NH_4^+ fixation. In order to explain the results, let us consider the following points —

(a) The soils treated by H_2O_2 were H+ saturated and therefore would be expected to fix more NH_4^+ (b) the presence of organic matter is known to interfere in ammonium fixation most probably by blocking [illegible] trance of NH_4^+ ions between the clay plates but the removal of organic matter by H_2O_2 treatment should favour an increase in NH_4^+ fixation [illegible] the clay (c) there is an increase in the % of clay due to treatment by H_2O_2 (Baver[4]) and (d) the decrease in the particle size of the clay [illegible] to exfoliation by H_2O_2 treatment should increase both the [illegible] and [illegible] tion of NH_4^+ (Barshad[7]) Taking all these facts into consideration [illegible]

quite obvious that if the clay fraction of the soil may be regarded as the main seat for NH_4^+ fixation, then there should be an increase in the fixation of NH_4^+ due to H_2O_2 treatment But the decrease in fixation of NH_4^+ as is evident from table 5 leads us to believe that the organic matter of the soils possess the capacity to fix NH_4^+ in nonexchangeable form

Summary

The effect of H_2O_2 on soil organic matter was studied and it was observed that the C and N % decreases as a result of this treatment The repeated treatments were found to be more effective than the single treatment. The loss of C and N was less in alkali soils as compared to normal one A decrease in the cation exchange capacity was also observed. The oxidation of soil organic matter was greatest in H-saturated soils followed by Na soils and least in Ca-soil The raw organic matter is not easily decomposed by H_2O_2. The fixation of NH_4^+ was found to decrease due to the removal of organic matter by H_2O_2.

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LONGEVITY OF *LINUM GRANDIFLORUM* Desf POLLEN AND COMPARISON OF THREE TESTS FOR POLLEN VIABILITY

D K. Dubey
B R. College Bichpuri (Agra)

The storage longevity of pollen grains is of great importance in present day breeding programme. Holman and Brubaker (1926) Visser (1955) and Brewbaker (1959) have reviewed the literature on prolongation of pollen viability by storing the pollen under various conditions. The storage conditions tried are low temperature (Nebel, 1939 Sartoris, 1942 Pfeiffer 1944 Jones and Newell, 1948 Visser 1955) and controlled humidity (Holman and Brubaker 1926 Ferwerda, 1937) Recently Singh (1960) has tried low temperature and controlled humidity simultaneously and found that 23·98% of papaya pollen were viable even after eleven months when stored in a desiccator at 25°C and with 0% R.H under the room conditions viability was lost within 24 days The present study on the longevity of *Linum grandiflorum Desf* pollen was taken up with a view to find out the duration for which the pollen remain viable under different storage conditions.

There are several distinct type reactions of pollen grain such as stainability germinability (in vitro) burstingability in certain mineral acids (Koul & Paliwal 1961) and fertilizing ability (or % seed-set) which are commonly utilized for testing the percentage of viable and non-viable pollen grains. Germinability and fertilizing ability out of the above methods are considered to be most reliable, whereas use of stainab lity as test of viability has recently been questioned by Vasil (1958) Singh (1960) and Johri and Vasil (1961) But as the methods of recording germination percentage and the percentage of seed-set are more time taking these are almost impracticable for the plant breeder who has to test a large number of samples within a comparatively short period. The mineral acid test, however seems to be a reliable one and Koul & Paliwal (1961) while testing the male sterile and male fertile lines of *Plantago ovata* have found it to work accurately In the present study on *Linum Grandiflorum Desf* two stains *i. e* iodine and acetocarmine and the mineral acid test were used for testing the pollen viabil ty with an idea to confirm whether the three chemicals give similar results o not by correlation studies

MATERIAL AND METHODS

The flowers of *Linum Grandiflorum* w th freshly dehisced anthers were collected and placed in three dry petridishes. Out of the three petridishes one, each was placed under the following three storage conditions —

(1) In a desiccator over calcium chloride (0% R H and 23 to 44°C room temp)

(2) In refrigerator (at 6°C & 100% R. H.)

(3) On the table inside laboratory (23 to 44°C room temp. and 69 to 87% R. H.)*

Percentage of viable pollen grains from the anthers of stored flowers was recorded after every 24 hours utilising the following three tests —

1. Iodine stain test.
2. Acetocarmine stain test.
3. Mineral Acid Test (25% HCl was used)

The viability percentage data obtained by the three tests was plotted against time, in form of three separate graphs, each representing one storage condition (Figs. 1, 2 and 3)

Results and Discussion

The viability of freshly collected *L. grandiflorum* pollen was found to be 92·52 per cent as revealed by iodine test, 95·04 per cent by acid test and 96·08 per cent by acetocarmine test. The viability of pollen preserved in different storage conditions was examined upto 22 days and results are summarised in the Table.

It is apparent from the Table that *L. grandiflorum* pollen stored under room conditions could retain viability only upto six days when only 1·4- 2·38% pollens were found to be viable whereas pollen preserved at room temperature inside a desiccator over calcium chloride showed 2·61-4·6% viability on the 9th day of storage. The sample preserved inside the refrigerator at 6°C and 100% R. H. gave the best results where viability was retained even upto 22 days as shown by viability test (1·97-3·91 viable pollens)

It thus appears that *L. grandiflorum* pollens do not completely lose their viability readily even at the room conditions as some of them could retain viability for six days. The longevity however could be prolonged by storing the pollen under cool conditions.

The time for which viability could be prolonged in *L. grandiflorum* is comparatively very short as compared to certain other crops for example papaya where Singh (1960) could keep the pollen viable for eleven months in a desiccator at 23°C. This difference in the retention of pollen viability is in confirmity with the statement of Brewbaker and Majumdar (1961) where they have mentioned that most binucleate grains store well (i.e. papaya) while trinucleate grains seldom retain viability for more than a few days as is seen in case of *Linum grandiflorum* during present study

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*Atmospheric temperature and humidity data obtained from Meteorological Observatory, Bichpuri Farm.

TABLE

Viability percentage of *Linum grandiflorum. Desf* pollen under various storage conditions, utilising three different tests.

Viability Percentage of Pollen grains	Pollen preserved under room conditions			Pollen preserved inside Desiccator (0% R H) at room temperature			Pollen preserved inside refrigerator at 6°C and 100% R H		
	Iodine Test	Mineral Acid Test	Acetocarmine Test	Iodine Test	Mineral Acid Test	Acetocarmine Test	Iodine Test	Mineral Acid Test	Acetocarmine Test
Fresh	92 52	95 04	96 08	92 52	95 04	96 08	92 52	95 01	96 08
Under storage for days									
One	78 26	77 97	88 25	63 49	69 70	62 51	92 50	94 76	95 52
Two	52 90	57 45	49 42	28 05	28 12	29 50	87 20	85 11	84 66
Three	42 61	51 52	48 62	21 15	23 74	21 83	87 20	85 03	81 85
Four	38 51	31 77	38 44	21 53	21 93	19 48	83 27	82 91	80 75
Five	21 58	17 59	19 44	18 95	20 39	19 30	80 33	80 45	79 71
Six	2 38	Nil	1 96	13 75	11 22	17 32	79 53	81 25	80 62
Seven	Nil		Nil	7 52	6 06	6 58	78 92	79 97	80 51
Eight				5 25	6 19	4 92	79 06	78 03	80 47
Nine				Nil	2 64	4 46	73 75	72 87	70 63
Ten					Nil	Nil	68 18	58 09	60 83
Eleven							60 24	58 45	59 22
Twelve							60 12	57 52	58 55

(Continued on page 72)

TABLE (*Contd.*)

Viability percentage of *Linum grandiflorum* Desf pollen under various storage conditions, utilizing three different tests.

Viability Percentage of Pollen grains	Pollen preserved under room conditions			Pollen preserved inside a Desiccator (0% R.H.) at room temperature			Pollen preserved inside a refrigerator at 6°C and 100% R.H.		
	Iodine Test	Mineral Acid Test	Acetocarmine Test	Iodine Test	Mineral Acid Test	Acetocarmine Test	Iodine Test	Mineral Acid Test	Acetocarmine Test
Thirteen	...	...	...	...		...	56 55	57 84	54 89
Fourteen	...	...	...	...	...		53 25	55 27	56 15
Fifteen	...	...			...	...	49 35	51 73	53 54
Sixteen	...	...	...	...			47 42	45 39	50 23
Seventeen	...	...	...	...	...	...	45 13	42 71	49 42
Eighteen	...	...	...		...	...	40 01	41 51	47 31
Nineteen			...	...	...		35 17	38 89	42 24
Twenty	...	...	...	...	...	...	21 23	27 37	23 97
Twenty-one	...		...		...	...	13 73	1 73	19 12
Twenty-two	...				...	...	2 25	1 97	3 13
Twenty-three	...	...		...	...	...	Nil	Nil	Nil

Correlation Study—A perusal of figures 1 2 & 3 shows that under all the three conditions of storage the curves of three different chemicals obtained by plotting the percentage viability against time adopt a similar path. At no point the curves show a deviation of more than 10 % which can be taken to be negligible, when the curves show a positive correlation between them. Thus it could be concluded that the results of pollen viability given by the three chemicals *i.e.* Iodine, Acetocarmine and Hydrochloric Acid seems to be quite reliable for practical purposes.

Summary

The longevity of pollen grains of *Linum grandiflorum D f* was tested under varying conditions and following results were obtained.

1 At room conditions *L. grandiflorum* pollen remained viable for six days and in a desiccator over calcium chloride it retained viability for a longer duration of nine days. B t the greatest prolongation of pollen viability was achieved when the pollens were stored inside a refrigerator (t 6°C) where they showed viability for twenty two days

2. The three chemicals *i e* Iodine, Acetocarmine and hydrochloric acid, utilised to test the viability of pollen grains gave similar results.

Acknowledgments

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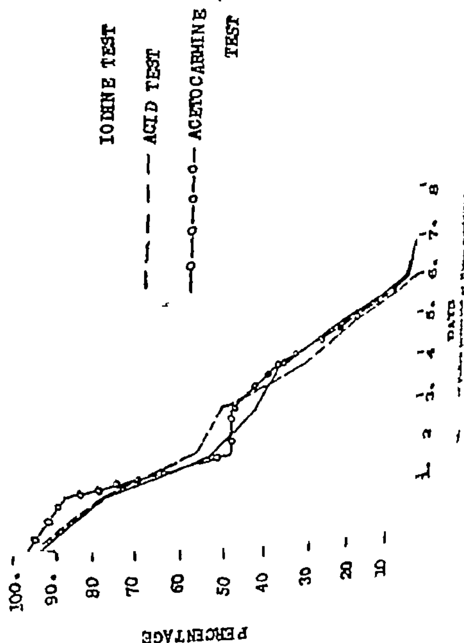
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LEGEND TO THE FIGURES

Figs 1-3 Graphs showing percentage of viable pollens under different storage conditions as shown by Iodine Acid and Aceto-carmine tests



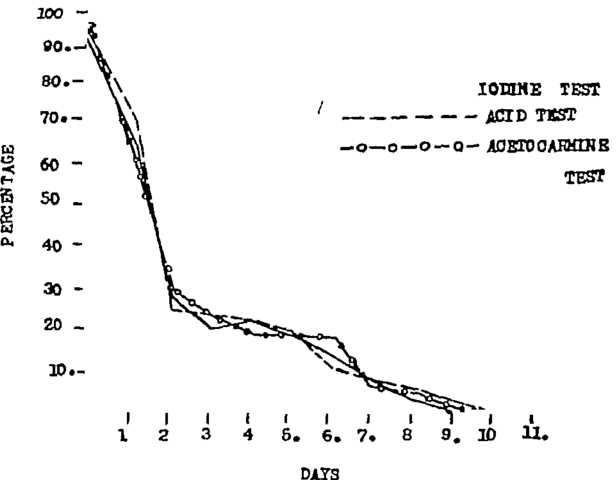


Fig. 2 For Pollen preserved in a Desiccator (Zero % R. H.) at Room Temperature.

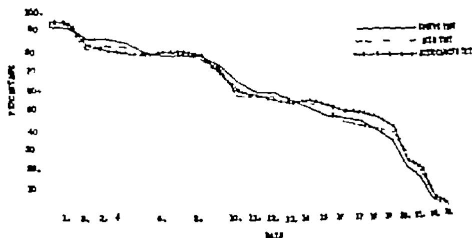


Fig. 3 F r Pollen preserved in Refrigerator (6°C and 100% R. H.)

MALONANILIC ACID HYDRAZIDE AS A REAGENT FOR CARBONYL COMPOUNDS

(KM) SUSAN GEORGE AND P I ITTYERAH
Chemistry Department, St John's College Agra

The importance of acid hydrazides in the field of medicine and in synthetic organic chemistry is well known. Earlier workers in this laboratory prepared a number of new acid hydrazides[1] Later on some of these hydrazides were tried as reagents for carbonyl compounds and the results obtained were published[2] The present paper deals with the use of malonanilic acid hydrazide as a reagent for aliphatic, aromatic and heterocyclic aldehydes and ketones

Malonanilic acid hydrazide was prepared according to the method described by Brijraj Singh Rathore and Ittyerah.[1] The melting point and other physical and chemical properties of the compound were found to be the same as reported by them. Several hydrazones have now been prepared using this acid hydrazide The general procedure has been to take a mixture of equimolecular quantities of the carbonyl compound and the acid hydrazide in ethanol and to keep the mixture at room temperature for fifteen to twenty minutes In some cases like crotonaldehyde, n heptaldehyde, and *p*-dimethyl-aminobenzaldehyde the mixture had to be kept for about four hours for the hydrazones to crystallise out With ketones the mixture had to be refluxed for two hours and then cooled.

The carbonyl compounds selected were benzaldehyde, m nitrobenzaldehyde, anisaldehyde piperonal *p*-dimethylaminobenzaldehyde, salicylaldehyde, 5-chloro 3 5-dichloro 5-bromo 3.5-dibromo 3.5-diiodo 3-nitro 5-nitro, 3.5-dinitro, and 5-chloro-3-nitro salicylaldehydes 2 thiophenaldehyde, chloral, n-heptaldehyde, crotonaldehyde methyl ethyl ketone, acetophenone and benzophenone. The only reason for choosing these particular carbonyl compounds was their easy availability in the laboratory at the time of investigation.

The nitro-salicylaldehydes gave yellow hydrazones but all the other hydrazones were white. The results obtained point to the possibility of using malonanilic acid hydrazide as a reagent for the characterisation of aldehydes and ketones

EXPERIMENTAL

Preparation of malonanilic acid hydrazide has already been described.[1] The substituted salicylaldehydes were prepared in the laboratory using well known methods described in literature. For obtaining the hydrazones the method employed was the same in most cases and as an example the preparation of the hydrazone of anisaldehyde is given below —

Malonanilic acid hydrazide (0 5g) and anisaldehyde (0.55g) were dissolved separately in 10 ml. each of ethanol. The solutions were then mixed and kept aside for fifteen minutes at room temperature. The hydrazone which separated was filtered recrystallised from alcohol and dried. It melted at 199° and the yield was 0 8g (Found N 16·88. $C_{17}H_{17}N_3O_3$ requires N, 17%)

As mentioned earlier the conditions had to be slightly altered for the preparation of the hydrazones of crotonaldehyde, n-heptaldehyde p-dimethylaminobenzaldehyde and the ketones The m.p yield etc. of the hydrazones are given in the accompanying Table

TABLE

Giving the m.p yield etc of the hydrazones

No.	Malonanilic acid hydrazone of:—	Mol Form	M P °C	Yield %	N % or Halogen % Req	N % or Halogen % Found
1	Benzaldehyde	$C_{16}H_{15}N_3O_2$	214	80	18.18	18.11
2	m-Nitrobenzaldehyde	$C_{16}H_{14}N_4O_4$	225	86	17.18	16.76
3	Anisaldehyde	$C_{17}H_{17}N_3O_3$	199	99	17.0	16.88
4	Piperonal	$C_{17}H_{15}N_3O_4$	204	45	13.54	19.[illegible]
5	p-Dimethylaminobenzaldehyde	$C_{18}H_{20}O_2N_4$	198	30	17.28	16.10
6	Salicylaldehyde	$C_{16}H_{15}N_3O_3$	253	82	14.14	14.12
7	5-Chloro-salicylaldehyde	$C_{16}H_{14}N_3O_3Cl$	219	93	10.71	11.30
8	3:5-Dichloro-salicylaldehyde	$C_{16}H_{13}N_3O_3Cl_2$	218	100	19.4	19.23
9	5-Bromo-salicylaldehyde	$C_{16}H_{14}N_3O_3Br$	212	83	21.27	21.25
10	3.5-Dibromo-Salicylaldehyde	$C_{16}H_{13}N_3O_3Br_2$	226	62	33.17	33.96
11	3:5-Diiodo-salicylaldehyde	$C_{16}H_{13}N_3O_3I_2$	232	93	46.26	46.03
12	5-Nitrosalicylald hyde	$C_{16}H_{14}N_4O_5$	256	95	27.18	16.94
13	3-Nitrosalicylaldehyd	$C_{16}H_{14}N_4O_5$	252	99	17.18	16.7
14	3:5-Dinitro-salicylaldehyd	$C_{16}H_{13}N_5O_7$	214	96	13.87	11.5
15	3-Nitro-5-chlorosalicylaldehyde	$C_{16}H_{13}N_4O_5Cl$	221	91	9.6	9.13
16	2 Thiophenaldehyde	$C_{14}H_{13}N_3O_2S$	203	59	11.13	13.9
17	Chloral	$C_{11}H_{10}N_3O_3Cl_3$	263	51	31.23	31.18
18	n-Heptaldehyde	$C_{16}H_{23}N_3O_2$	145	44	11.33	11.52
19	Crotonaldehyde	$C_{13}H_{15}N_3O_2$	170	37	17.14	15.13
20	Methyl ethyl ketone	$C_{13}H_{17}N_3O_2$	155	43	17.0	15.1
21	Acetophenone	$C_{17}H_{17}N_3O_2$	212	53	14.23	11.6
22	Benzophenone	$C_{22}H_{19}N_3O_2$	185	37	11.6	11.8

Note—Halogen or sulphur was estimated in compounds which contain [illegible] of these and nitrogen in the rest

Summary

Malonanilic acid hydrazide was prepared and using this malonanilic acid hydrazones of benzaldehyde, *m*-nitrobenzaldehyde, anisaldehyde, piperonal, *p*-dimethylaminobenzaldehyde, salicylaldehyde, 5 chloro, 3.5-dichloro, 5-bromo, 3.5-dibromo, 3.5-diiodo, 3-nitro, 5-nitro, 3.5-dinitro and 5-chloro-3-nitro salicylaldehydes 2-thiophenaldehyde, chloral *n*-heptaldehyde crotonaldehyde, methyl ethyl ketone, acetophenone and benzophenone have been prepared. It has been found that malonanilic acid hydrazide is a good reagent for the detection identification and characterisation of aliphatic aromatic and heterocyclic aldehydes and ketones.

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MALON-p-IODOANILIC ACID AND SOME OF ITS DERIVATIVES

B. P. ASTHANA AND P. I. ITTYERAH
Chemistry Department, St. John's College, Agra

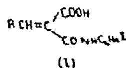
A large number of workers have studied the reaction between primary aromatic amines and ethyl malonate. To mention only a few Freund[a], Rugheimer and Hoffmann[a], Whitely[a], Chattaway and Mason[a], Chattaway and Olmsted[a] and Ahluwalia, Haq and Ray[1] have done considerable work in this line. In this laboratory Chellappa and Ittyerah[4] prepared malon 1,3,4 xylidic acid by condensing freshly distilled 1,3,4- xylidine with pure ethyl malonate. Again George and Ittyerah[a] have prepared malon-o-,m and p-chloranilic acid from malonic ester and the corresponding chloroaniline.

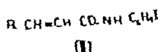
In this paper is described the preparation of malon-p-iodoanilic acid and its reaction with certain aldehydes.

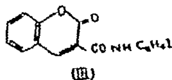
When ethyl malonate is allowed to react with p-iodoaniline, two products can be expected (a) ethyl-malon-p-iodoanilate and (b) malon-di-p-iodoanilide. Both these products have been obtained in the course of the present investigation. No attempt has been made to isolate in pure state the ester (a) which on hydrolysis yielded malon-p-iodoanilic acid. Malon-p-iodoanilic acid melted with decomposition at 162° and malon-di-p-iodoanilide melted at 256°. The maximum yield of the acid was approximately 64%.

It was observed that in some experiments when ethyl malonate and p-iodoaniline were mixed and warmed the mixture turned dark brown due to the evolution of iodine and the expected reaction did not take place. On investigation it was found that this was caused by impurities present in the starting materials and to overcome this difficulty very pure materials had to be used. It must also be mentioned that the duration and rate of heating also affected the purity and yield of the products.

Malon-p-iodoanilic acid was condensed with benzaldehyde, o-chlorobenzaldehyde, 2 thiophenaldehyde, salicylaldehyde and 3,5-dichlorosalicylaldehyde. With the first three aldehydes the products obtained were the corresponding arylidene-malon-p-iodoanilic acid (I) and the corresponding cinnam-p-iodoanilide (II). With salicylaldehyde the product was mainly coumarin 3-







carboxy-p-iodoanilide (III). Along with this coumarin derivative some salicyl-

al-*p*-iodoaniline was also obtained The formation of this Schiff's base seems to be a peculiar feature of the *o*-hydroxy-aldehyde condensations alone. This observation has been made in this laboratory by some earlier workers also. The identity of this product was established both by analysis and by mixed melting point determination with an authentic specimen prepared by the condensation of salicylaldehyde and *p*-iodoaniline. With 3,5-dichlorosalicylaldehyde the expected coumarin derivative was not obtained under the conditions tried. The only product obtained was 3 5-dichloro-salicylal-*p* iodoaniline m.p. 19.

The condensations were carried out both in the presence and in the absence of condensing agents. The condensing agents used were glacial acetic acid pyridine and piperidine. Piperidine was found harmful in these condensations as it produced only resinous products from which no pure compound could be isolated. The other two condensing agents catalysed the reaction

Attempts to condense malon-di-*p*-iodoanilide with benzaldehyde were unsuccessful

EXPERIMENTAL

Malon-p-iodoanilic acid and malon-di-p-iodoanilide

A mixture of freshly crystallised *p*-iodoaniline (5 g) and freshly distilled ethyl malonate (8 g) was taken in a 100 ml. round bottomed flask fitted with a condenser of such a length that the alcohol formed during the reaction escaped whil the ethyl malonate condensed and flowed back. The mixture was gently boiled for an hour After cooling ethanol (10 ml) was added and the mixture stirred vigorously The crystalline precipitate formed was filtered and recrystallised from absolute alcohol. The colourless needles (1 7 g) of malon-di *p* iodoanilide thus obtained melted at 2 (Found N 5·08 $C_{15}H_{12}O_2N_2I_2$ requires N 5 53 %)

Malon-di-*p* iodoanilide when exposed to air and light developed a tint.

The filtrate was then mixed with sodium carbonate (3g) dissolved in distilled water (40 ml) and steam was blown through it for an hour. The solution on cooling deposited a little more of the dianilide This was filtered off and the clear filtrate acidified with conc hydrochloric acid The crystalline precipitate formed was filtered and recrystallised from al The yield was on analysis was found to be malon *p*-iodoanilic acid. The and it melted with decomposition at 162° (Found N 4.21 $C_9H_8O_3NI$ requires N 4 59%)

In experiments where the duration of heating was shorter (minutes) the yield of both malon-di-*p*-iodoanilide and malon-*p*-iodoanilic acid was lower and heating for more than one hour resulted in the formation of much resinous matter

Benzylidene-malon-p-iodoanilic acid and cinnam-p-iodoanilide

Malon-*p*-iodoanilic acid (2 g) and benzaldehyde (0 7g) were mixed and heated on a steam bath. The mixture first melted to a clear yellow liquid but soon set to a solid After heating for four hours the contents were extracted with a warm solution of sodium carbonate in which the entire mass dissolved. This alkaline solution was then extracted with ether to remove all unreacted aldehyde. The alkali extract on addition of excess of conc. hydrochloric acid deposited 1 7g of benzylidene-malon-*p*-iodoanilic acid which after recrystallisation from aqueous ethanol melted with decomposition at 214 (Found N 3 67 $C_{16}H_{12}O_3NI$ requires N 3.56%)

The above experiment was repeated using a trace of pyridine (0 1 ml) as a condensing agent. On extraction with sodium carbonate solution a residue was left which on crystallisation from dilute ethanol gave 1.55g of cinnam-*p*-iodoanilide, m.p 190° (Found N 4.22 $C_{15}H_{12}ONI$ requires N 4.01%)

When piperidine was used in place of pyridine the whole mass resinified and on using glacial acetic acid as the condensing agent benzylidene-malon-*p* iodoanilic acid was the only product.

2 Thenal-malon-p-iodoanilic acid and 2-thienyl-p-acryl-p-iodoanilide

A mixture of malon-*p*-iodoanilic acid (2g) and 2-thiophenaldehyde (0 75 g) was heated for four hours on a steam bath and the resulting mass on extraction with a strong solution of sodium bicarbonate left a residue which on recrystallisation from dilute alcohol melted at 197 This was identified to be 2 thienyl-acryl-p-iodoanilide. The yeild was 0 4 g (Found S, 8.8 $C_{13}H_{10}ONSI$ requires S 9 01%)

The alkali extract on addition of conc. hydrochloric acid deposited 1.2 g of -thenal-malon-*p*-iodoanilic acid which after recrystallisation from aqueous ethanol melted with decomposition at 224° (Found S 7 71 $C_{14}H_{10}O_3NSI$ requires S 8.02%)

o-Chlorobenzylidene-malon-p-iodoanilic acid and o-chlorocinnam-p-iodoanilide

Malon-*p*-iodoanilic acid (2 g) and *o*-chlorobenzaldehyde (0.75 g) were mixed and heated on a steam bath for four hours. The mass first melted with slight effervescence but gradually solidified On extraction with a saturated solution of sodium bicarbonate in the usual manner a residue was obtained which after recrystallisation from aqeous alcohol gave *o*-chlorocinnam-*p*-iodoanilide (0.3 g) m.p 186° (Found N 3 59 $C_{15}H_{11}ONClI$ requires N 3.65%)

The alkali extract on addition of excess of conc. hydrochloric acid yielded white crystals of *o*-chlorobenzylidene-malon-*p*-iodoanilic acid (1 45g) which

after recrystallisation melted at 205 (Found N 3 05 $C_{16}H_{11}O_3NClI$ requires N 3 27%)

With a trace of pyridine as the condensing agent the yield of p-chlorocinnam *p*-iodoanilide was higher (1 72 g) whereas the yield of the acid product was low On the other hand when glacial acetic acid was used only the acid product (1.92 g) was obtained. Piperidine did not help as only resins were formed.

Coumarin-3-carboxy-p-iodoanilide and salicylal-p-iodoaniline

Salicylaldehyde (0 8 g) and malon-*p*-iodoanilic acid (2 g.) and a drop of pyridine were heated on the steam bath for four hours. The orange yellow mass was then treated with sodium bicarbonate but there was no reaction. The residue on fractional crystallisation using alcohol gave salicylal-*p*-iodoaniline (0 4 g) and coumarin-3-carboxy-*p*-iodoanilide (0·6 g) The former after recrystallisation from ethanol melted at 131° (Found N 4 35 $C_{13}H_{10}ONI$ requires N 4 33%) The identity of this compound was further established by preparing an authentic specimen by the condensation of salicylaldehyde with *p*-iodoaniline.

The latter after recrystallisation from ethanol melted at 230°. (Found N 4 14 $C_{16}H_{10}O_3NI$ requires N 3.84%)

Condensation of 3.5-dichloro-salicylaldehyde with malon-p-iodoanilic acid

Several attempts were made to condense the aldehyde and the acid but in all cases only 3.5-dichlorosalicylal-*p*-iodoaniline, m. p.190° was the only product obtained The identity of the compound was established by mixed melting point determination with an authentic specimen prepared by the condensation of the aldehyde with *p*-iodoaniline.

SUMMARY

Malon-*p*-iodoanilic acid and malon-di-*p*-iodoanilide were prepared by the action of ethyl malonate on *p*-iodoaniline. Very pure starting materials were found necessary as otherwise the amine decomposed. Malon-*p*-iodoanilic acid was then condensed with benzaldehyde *p*-chlorobenzaldehyde, salicylaldehyde and 3 5-dichloro-salicylaldehyde. The first two aldehydes gave the corresponding arylidene-malon *p*-iodoanilic acids and cinnam-*p*-iodoanilides. Salicylaldehyde gave Coumarin-3-carboxy-*p*-iodoanilide and salicylal-*p*-iodoaniline. 3.5-Dichloro-salicylaldehyde gave only 3.5-dichloro-salicylal *p*-iodoaniline The influence of glacial acetic acid pyridine and piperidine as condensing agents was also studied.

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PRODUCTION OF TWO SCAPES FROM A VARIETY OF *MUSA PARADISIACA* L.*

THOMAS M. VARGHESE
School of Plant Morphology, Meerut College, Meerut

The characteristic inflorescence of *Musa paradisiaca* L. is a solitary scape, which is a prolongation of the axial apex. The inflorescence emerges through the pseudostem which is formed by the sheathing bases of radical leaves all round the central axis.

A number of teratological variations in the inflorescence of *Musa* have been reported. Parija (1931) has described an abnormal banana plant with 8 inflorescences, in which one was terminal and the remaining were borne by branches from the last three penultimate nodes. All of them were sterile. Jacob (1938) observed the so called *fasciated inflorescence* in 'a banana variety *Kali* locally known as Mara bala (*Musa paradisiaca* L. var *Kali*) produced a dozen different 'hearts' (cone like inflorescence) of pistillate flowers developing into innumerable diminutive fruits while the original staminate 'heart' remained as such'. Davis (1948) recorded a *Musa* variety *pepen*, with four forkings of which three bore normal fertile fruits while the fourth one was sterile and minute. Ghosh and Chakravorty (1949) found a *Musa* plant which produced "as many as five inflorescences in addition to the normal and apparently terminal ones, at different levels on its trunk'. The accessory inflorescence were produced from the axils of leaves at the nodes'.

The present report deals with an interesting abnormality in a table variety of *Musa paradisiaca*, locally known as 'Chingan'. The plant was growing in Ranni a village in Kerala State. This plant exhibited some remarkable deviations from the previously reported abnormalities.

The sucker of this abnormal plant obtained from a mother plant which produced a very heavy bunch weighing about a hundred and ten pounds and the fruits were about double the size of normal ones of this variety. At maturity the abnormal plant produced one inflorescence to begin with. The height of the plant at that time was about eight feet. The leaves were much crowded and the plant resembled a diseased one, which was suspected as a symptom of the virus disease 'bunchy top' which is quite prevalent in Kerala. But the growth of the plant continued for about a foot more and produced another 'heart' ten days after the production of the first one. Thus the plant at maturity carried two 'hearts' of which the first was outwardly "axillary" while the second was terminal (see the Plate). Both 'hearts'

*Research contribution No. 52 from the School of Plant Morphology, Meerut College, Meerut, India.

had separate shot leaves and at the time of appearance of both the hearts, the plant had seventeen fresh leaves including the shot leaves. Both the hearts bore fruits of normal size. The 'hands' were equally spaced. The fruits produced from the upper (second) terminal inflorescence were a little bigger than those produced from the first one which had fruits of normal size.

At maturity the pseudo-stem was dissected and the leaf sheaths were removed one by one. It was noted that the inflorescence stalks maintained their individuality except at the basal portion. Each of the inflorescence stalks had four separate leaf sheaths forming its envelopes. Outside these there were a few leaf sheaths forming a common covering for both, giving the appearance of a single plant (see the text figure). Both the 'plants' were fused at the base for a few inches. The scapes were actually borne by two independent axes. The pseudo-stem externally exhibited no difference in form or size from the normal one. The transverse section of the base of the pseudostem (about 12.5 centimeters above the root-stock) was oval in shape. A little above this a groove appeared marking the limits of the two pseudo-stems. The abnormal plant noted by the author had the following peculiarities

(1) Inside some common sheaths there were two separate axes that showed fusion for a distance of about a few centimeters at the base.

(2) The hearts were produced at two different heights, so that one appeared terminal and the other axillary.

(3) The two scapes appeared at different times, the terminal inflorescence being younger by 10 days than the pseudo-axillary one.

Although several abnormalities with regard to the production of more than one inflorescence in *Musa* are reported, only a few authors (Davis, 1948; Ghosh and Chakravorty 1949) have attempted an interpretation of such abnormalities. Davis (1948) says "Probably due to the external stimulation or disturbances the meristematic tissue of the peduncle in very young stage might have been hurt, and as the result of which it might have continued growth with different growing points (apical meristem). Till the inflorescence has emerged the growth in different directions was not possible as the compact leaf sheaths do not give room for such lateral growth. Ghosh and Chakravorty (1949) dissected the abnormal plant noted by them and found that the accessory inflorescences were produced by the activation of dormant buds at the nodes.

There are three possible explanations of the abnormality reported here.

(1) The single apical meristem after giving rise to a few leaves, got divided by a longitudinal split. Thus two apical innovations (growing points) were formed which produced two separate axes while the first-formed leaves of the plant produced the common envelope through which the [illegible] meristems continued their growth separately up to maturity O[illegible]

these, one matured earlier and produced an inflorescence which occupied a 'pseudo-axillary' position while the other one continued its growth and produced a 'heart' at a higher level that appeared terminal'

(2) Two root stocks might have fused in a very young stage and given rise to some leaves which formed a common envelope by their sheaths. After growing for a short distance together they regained their individuality and produced two separate axes inside the first formed pseudostem.

(3) The production of an axillary bud in the axil of a leaf sheath and its subsequent development into an axis may lead the abnormal production

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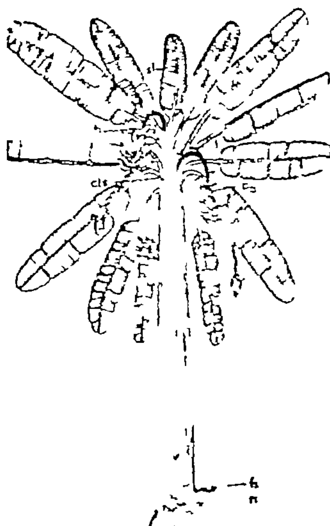
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Description of the Figure [illegible]

Diagrammatic representation of [illegible]

clsh — common leaf-sheaths

[illegible] — fused portion of [illegible]

— individual leaf-sheath

[illegible] — Pseudo-axillary inflorescence

[illegible] [illegible] stock

— leaf

— terminal inflorescence



A banana plant (*Musa Paradisiaca* L.) with two inflorescences.

EFFICIENCY OF DETERGENTS BY FARADAIC RECTIFICATION METHOD

H P AGARWAL*
Department of Chemistry
Motilal Vigyan Mahavidyalaya, Bhopal India.

INTRODUCTION

Surface active substances are known to affect the characteristic of polarographic waves. With the increase in concentration of the surface active substance the waves generally become flatter and are shifted towards more negative potential, except in some cases the wave is splitted into two waves.[1] This behaviour has been explained on the basis of adsorption of the surface active substance at the electrode or to its adherence with the reducible substance. Besides this, the surface active substances markedly influence the kinetics of electrode processes. It is in order to throw some light on this matter and to determine therefrom the efficiency of a few surface active substances the faradaic rectification method has been used The method has been found to be very convenient and useful[2] for the study of fast electrode processes and it is based on observing the shift in mean potential when an electrode is being polarised with a sinusoidal current.

EXPERIMENTAL

Reagents

The calculated amounts of ferric ammonium sulphate (A. R.) and ferrous ammonium sulphate (A.R) (concentration of Fe^{++} and Fe^{+++} each being 002 M) were dissolved in 1 N sulphuric acid (A.R.) containing a fixed amount of the detergent. Lissapol N (I.C.I) Cetyl pyridinium bromide-Fixanol C (I.C.I.) and Teepol (neutral lab reagent B.D H.) were chosen for the study

Procedure

The earlier experimental cell[3] used for the measurement of redoxokinetic potential was suitably modified[4] so that the two a.c. electrodes may be separated from each other in such a manner that there may be a uniform distribution of current density The R drop in between the reference electrode (third electrode) and the polarised (measuring) electrode was avoided by keeping the two close together within a distance of 5 cm All the data were obtained for Platinum/aqueous solutions The magnitude of a.c. voltage between the reference electrode and polarised electrode was measured by connecting them (electrodes) to th Y-axis of a high sensitive Philips Oscillograph type GM 3156 whose sensitivity was 1 mv (r m s) per cm. A Philips audio oscillator type GM 2315 served as an a.c. source. The technique for measuring the shift in mean potential was also improved The rectified d.c.

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*Present Address—Professor of Chemistry M. A. College of Technology Bhopal.

potentials were measured[8] potentiometrically after filtering off a.c. through low frequency pass filters. The potentiometer used could measure correctly potentials of the order of 1 micro volt using a high sensitive Pye & Co moving coil mirror galvanometer, Resistance = 1840 Ohm, sensitivity 4.5mm/μv at a distance of 1.1 meter. The observations were taken at 4 mv and 8 mv of alternating voltage so as to verify the variation of square of a.c. voltage V with the Redoxokinetic potential, Ψ. The results obtained are given in table 1 except that the values of Ψ at 8 mv have not been given, as they were found to be exactly 4 times to those obtained at 4 mv.

In presence of .002% of cetyl pyridinium bromide (fixanol C) the diffusion coefficient of .001 M ferric ammonium sulphate in 1 N sulphuric acid was determined by the fritted glass porous diaphragm cell method. The amount of ferric salt diffused in the other compartment of the cell was determined by the electro-chemical method whose details are given in a separate communication. The values of concentration gradient and of mean concentration for diffusion of .001 M ferric salt in 1 N sulphuric acid containing .002% solution of cetyl pyridinium bromide (fixanol C) are given in table 2.

RESULTS AND DISCUSSION

On addition of lissapol N and fixanol C to the ferrous ferric redox couple, Ψ, the redoxo-kinetic potential is found to decrease in magnitude at all frequencies as compared to the values obtained (26 μv at 500 cycles sec. or above) when the detergents are not being added[8] to the system. When teepol is being added to the redox reaction, similar results are obtained except that at a frequency of 200 cycles per sec. or below the shift in mean potential becomes positive (because the decrease in negative values leads to more positive values). In all the three cases Ψ becomes constant at a frequency of 1000 cycles per sec. or above in agreement with the theoretical analysis.[6] Below a frequency of 500 cycles per sec. Ψ varies with the square root of frequency when fixanol C and lissapol N are being used, but with teepol the relationship does not hold true. It may be noted that lissapol N does not form truely homogeneous solution, (turbid solution) as after allowing the solution to stand for more than 40 hours fine particles settled at the bottom of the cell.

On applying the theoretical equation[6] at high frequency

$$\alpha = 0.5 - \frac{2\Psi RT}{V^2 nF} \quad \quad (i)$$

(where α is the transfer coefficient, R is the gas constant, T is the absolute temperature, n is the valency, V is a.c. voltage incident and F is the Faraday), the transfer coefficient in presence of different surface active substances can be calculated by substituting V = 4 mv and Ψ at a frequency of 1000 cycles/sec. or above from table 1. The values of α thus obtained a

.373 with lissapol N .566 in presence of fixanol C (cetyl pyridinium bromide) and .526 when teepol is being added to the ferrous ferric redox reaction. In absence[3] of surface active substances, the transfer coefficient of ferrous-ferric redox couple is .586. It may therefore, be concluded that on addition of above detergents the transfer coefficient decreases. The lowering of transfer coefficient indicates that the rate of reduction during the cathodic half wave is retarded in presence of the surface active substances probably due to their adsorption at the electrode surfaces. The extent of decrease in transfer coefficient may be taken as a measure of detergent action of the surface-active substance, and for the substances tried the efficiency varies in the following order

Teepol > Fixanol C (Cetyl pyridinium bromide) > Lissapol N

For seeing the influence of detergents on rate constant, it becomes necessary to determine diffusion coefficient of the reactants when the surface-active substance is present in the supporting electrolyte. The diffusion coefficient of ferric ammonium sulphate under the above conditions was calculated from the data given in table 2 by following the method of Hartley and Runnicles.[7] The diffusion coefficient of ferric salt in 1 N sulphuric acid containing ·002% fixanol C (cetyl pyridinium bromide) thus obtained is 2.0×10^{-5} cm.^{2} sec.$^{-1}$ The value of rate constant when fixanol C is being added to the redox reaction was calculated by making use of the theoretical equation[4] applicable at lower frequency i.e.

$$K = (5-\alpha)\frac{V^2 nF}{4\Psi RT}\sqrt{\frac{\omega D}{2}} \quad \ldots \quad \ldots \quad (ii)$$

where ω is the angular frequency ($\omega = 2\pi f$, f is the frequency of a.c. in cycles/sec.) D is the diffusion coefficient, K_s is the rate constant, and the other notations are the same as given for equation (i) The transfer coefficient obtained at .002 M concentration of the reactants is the same[3] as that obtained with .001 M concentration Hence substituting in equation (ii) the value of α = .566 (obtained when .002% fixanol C is added to the reactants.) the value of Ψ redoxokinetic potential, at a frequency of 200 cycles/sec. at 4 mv of a.c. from table 1 and the value of diffusion coefficient D as given above, the value of K_s thus obtained is 12 cm sec.$^{-1}$ On comparing this value with that of ferrous and ferric redox couple (concentration of each cation is 001 M in 1-N H_2SO_4) alone[4] (K = .045) it is found to be 2.5 times higher The increase in rate constant may perhaps be due to the forming of a complex in between the surfactant and the reactants.

Summary

On addition of surface active substances the transfer coefficient of ferrous-ferric redox system decreases, indicating thereby that the reduction during the cathodic half wave is favoured less in their presence. The transfer coefficients of ferrous-ferric redox couple in presence of lissapol N

fixanol C (cetyl pyridinium bromide) and teepol neutral are ·573 ·506 and ·526 respectively The extent of decrease of transfer coefficient is a measure of the detergent action of the surface active substance. The rate constant is also tremendously influenced on addition of fixanol C. The value of rate constant determined for ·001 M Fe^{++} ·001 M Fe^{+++} in 1 N sulphuric acid containing ·002% fixanol C (cetyl pyridinium bromide) is 0 12 cm. sec^{-1}

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Table 1

Dimensions of bright polished, platinum foil electrodes used —

a c electrodes	*length in cms*	*breadth in cms*
1	1 50	1 15
2	1 50	1 10 (earthed)
Reference electrode	1.50	1 10

Temperature of the thermostat 35° ± .05°C

Serial No.	a.c. frequency in cycles per second	Values of Ψ redoxokinetic potential in microvolts at 4 mv of a c when concentrations of oxidant and reductant (Fe^{++} and Fe^{+++}) each is ·001 M in 1 N H_2SO_4 containing —		
		2% solution of Lissapol N	·002% solution of Fixanol C	[illegible]
1	50	−9	−6	+15
2.	100	−12	−9	+6
3	200	−16	−12	−5
4	500	−20	−14	−5
5	1000	−22	−20	−9
6	2000	−22	−20	−8
7	3000	−22	−20	

TABLE 2

Values of concentration gradient and of mean concentration for diffusion of 001 M ferric ammonium sulphate in 1 N H_2SO_4 containing 002 % solution of fixanol C

Volume of the ferric ammonium sulphate solution taken in compartment A of the porous diaphragm cell = 60 c. c.

Volume of the supporting electrolyte (002 % solution of fixanol C in 1 N H_2SO_4) taken in compartment B of the porous diaphragm cell = 110 c.c

Characteristic constant of the cell = 3 46

Temperature of thermostat = 35° ± 05°C

Time t_1 in minutes.	Concentration of the diffused salt in gm. Equ/litre. $C_1 \times 10^4$	Time t_2 in minutes.	Concentration of the diffused salt in gm Equ/litre. $C_2 \times 10^4$	Concentration gradient. $\frac{dc}{dt} \times 10^4$	Mean concentration. $\overline{C} \times 10^4$
308	36 5	699	63 1	067	49 7
345	39 8	668	61 6	067	50 7
377	43 6	638	58 9	06	51.2
419	45 7	608	56 9	06	51.3
455	49 3	578	54 9	045	52 1
505	50 7	547	52 5	045	51.6
			Mean	06	51.0

AMMONIUM FIXATION IN SOILS

• A. N Pathak and H. S Srivastava
Agricultural Chemistry Section, Govt Agricultural College Kanpur

Early in 1915 Ruprecht and Morse claimed the absorption of ammonium nitrogen from ammonium salts in soils through base-exchange phenomenon, which was also supported by Miyake (1916) At that time there was no idea of ammonium retention or fixation along with its absorption. NH_4 fixation in soils was brought into prominence in 1917 by Mc Beth who showed that under most condition some soils fixed applied NH_4 in a form such that it can not be removed by alkaline distillation or extraction with 10 per cent HCl. Later on Subramanyan (1924) Porges (1929) Chaminade and Drouneau (1936) Buswell and Dudenbostel (1946) Mettson and Anderson (1942) Cornet (1945) Levine (1916) and Jackson and Chang (1947) have presented evidences of NH_4 accumulation in soils as a result of fixation.

Bower (1950) found that Na^+ under certain circumstances is somewhat more effective than K^+ in displacing fixed NH_4^+ ions in clay minerals. Barthad (1951) has shown his simplest method of determining fixed NH_4^+ by saturating duplicate samples with NH_4^+ and distilling one sample with NaOH and the other with KOH The difference in ammonium replaced would equal to the fixation capacity for NH^+ Mortland (1955) Allison and Roller (1955) Harway and Scott (1956) Nash and Marshall (1956) and Govinda Rajan and Venkata Rao (1957) have reported that clay predominant minerals like Vermiculite, Illite montmorillonite, biotite and even feldspar have the capacity to fix considerable NH_4 ions.

Mc Dowell and Smith (1958) reported that soil texture has a pronounced effect on NH_4^+ movement and retention Recently Sohn and Peech (1958) Dhariwal and Stevenson (1958) Goring and Martin (1959) Scott Hunziker and Hanway (1960) Axley and Legg (1960) and Walsh and Murdock (1960) have studied the different aspects of the soil viz Clay content organic matter exchange capacity and clay minerals in relation to NH^+ fixation.

Material and Method

The experiment was conducted with a normal cultivated and uncultivated soils and usar soil. The cultivated and uncultivated soils were taken from the students Instructional Farm, Govt. Agricultural College Kanpur (U P) and the Usar soil was taken adjacent to the farm near the Allen Forest. Sufficient amount of soils, each was saturated with NH_4 by the N ammonium acetate method and free ammonium acetate was washed out by dil alcohol The soil was subsequently divided nto seven parts and treated as follows

One part was digested as usual for total nitrogen to determine the adsorbed NH_4 N as well as total nitrogen. Five parts were distilled each with a different bases like MgO NaOH KOH $Ba(OH)_2$ and $Ca(OH)_2$ and the amount of NH_4 was determined in the distillate to see the relative effectiveness of bases. The seventh part was leached with N $BaCl_2$ and the amount of NH_4 was determined in the leachate to see the relative effectiveness of leaching and distillation. Ammonium fixing capacity of different fractions from these soils was also studied.

Cation exchange capacity of the soils was taken as the difference between the total nitrogen of the untreated soil and total nitrogen of NH_4^+-saturated soil. The NH_4 fixing capacity was taken as the difference between the cation exchange capacity of the soil and the replaceable ammonium as determined by distillation with different base in Parnas Wagner micro distillation unit.

Experimental Results

From the data in table 1 it is observed that not all the NH_4 that enters the exchange complex is replaced by either of the extractants used. Some portion of the NH_4 that has entered the exchange complex is fixed and is not extracted. However in normal cultivated and uncultivated soils MgO distillation gave out the maximum amount of replaceable NH_4 whereas in Usar soil NaOH distillation gave out the maximum amount. It is further observed that Ba is more effective in replacing adsorbed NH_4 by distillation than by leaching. This might be due to the expansion of the lattice of clay minerals (which had adsorbed NH_4 on their surface) and by the po... effects of heating at the time of distillation. The other extractants like KOH and $Ca(OH)_2$ were not so effective.

Attempts were then made to determine the NH_4 fixing power of the different fractions of the soil and their contribution in fixation. The data are incorporated in tabl 2 and 3.

From the data in table 2 it is observed that clay fixed highest amount of NH_4. Allison Kefauver and Roller (1953) also observed a similar phenomenon. Sand invariably fixed more NH_4 than silt which falls in line with the observation of Coroll (1931-32). Hosking (1948) and Barshad (1951) and can be attributed to the association of some clay minerals with the sand particles.

It is interesting to note from the data in table 3 that NH_4 fixing capacity of soil as found by the actual analysis is in close proximity to the sum calculated of the different fractions. This falls in line with the observations of Pathak Mukerji and Shrikhande (1949).

General Discussion

Various mechanisms have been put forward regarding ammonium fixation in soils. One mechanism results from a replacement by ...

TABLE I

Ammonium Fixation in Soils

(Taking exchange capacity of the soil on digestion of the soil for total nitrogen)

Method of replacing ammonium from soil	Nature of the adsorbed ammonium	Usar			Uncultivated			Cultivated		
		0—6"	6—1	1—2	0—6"	6"—1	1—2	0—6	6"—1	1—2
Total nitrogen of Soil m.e %		2 5	3 0	1 3	7 1	1 3	4 0	3 7	3 2	2 9
1 Total nitrogen of soil saturated with NH_4 m. e. %		10 8	12 3	8 8	23 7	21 2	17 8	17 2	20 6	24 6
	Exchange capacity for ammonium m. %	8 3	9 3	7 3	15 6	16 9	13 8	13 5	17 4	21 7
2 Dist. With MgO	Replaceable m. %	6 6	8 1	4 9	13 3	14 5	12 0	12 1	13 7	17 7
	Fixed — m. %	1 7	1 2	2 4	2 1	2 4	1 8	1 1	1 7	4 0
	Fixed — % of total	20 4	12 9	32 8	13 8	10 4	10 0	10 3	9 8	18 4
3 Dist. with N NaOH	Replaceable m. %	7 0	7 9	6 5	13 4	13 9	11 8	11 1	13 8	17 1
	Fixed — m. %	1 3	1 4	0 8	2 2	3 0	2 0	2 1	3 6	4 6
	Fixed — % of total	15 6	15 0	12 2	14 1	17 7	11 8	15 3	20 4	1 6
4 Dist. with N KOH	Replaceable m. e.%	6 5	7 7	5 2	12 8	12 6	10 7	11 2	12 7	16 0
	Fixed — m. %	1 8	1 6	2 1	2 8	4 3	3 1	2 3	4 7	5 7
	Fixed — % of total	21 6	17 2	28 6	17 9	25 4	22 3	17 0	26 6	26 2

(Continued on Page 102)

TABLE 1 (*Contd.*)

Ammonium Fixation in soils

(Taking exchange capacity of the soil on digestion of the soil for total nitrogen)

Method of replacing ammonium from soil	Nature of the adsorbed ammonium	Usar			Uncultivated			Cultivated		
		0—6"	6"—1	1—2	0—6%	6—1	1—2	0—6	6"—1	1—2
5 Dist with $Ba(OH)_2$	Replaceable m. %	6 9	7 8	5 4	13 3	13 4	11 1	11 9	13 6	16 6
	Fixed — m. %	1 4	1 5	1 9	2 3	3 6	2 7	1 6	3 8	5 1
	Fixed — % of total	10 8	16 1	26 0	16 6	20 7	19 4	11 8	20 9	23 4
6 Dist with $Ca(OH)_2$	Replaceable m. %	6 5	6 0	5 9	12 0	12 1	7 9	11 4	12 2	14 0
	Fixed — m. %	1 8	2 3	2 4	2 7	4 8	5 9	2 1	5 2	6 8
	Fixed — % of total	21 0	26 8	32 8	17 3	28 4	42 4	15 5	20 4	31 2
7 Leaching with N $BaCl_2$	Replaceable m.	6 8	6 7	5 0	12 4	12 5	9 7	9 9	8 0	9 7
	Fixed — m.	1 5	2 6	2 3	3 2	4 4	4 1	5 6	6 6	12 0
	Fixed — % of total	18 0	27 9	31 4	20 1	20 0	~9 5	20 6	49 9	55 2

TABLE 2

Ammonium fixation in the various fractions of the soil

(Taking exchange capacity of the soil on digestion of the soil for total nitrogen)

Method of replacing ammonium from soil	Nature of th absorbed ammonium	Usar (0-6")			Uncultivated (0-6")			Cultivated (0-6")		
		Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
Total nitrogen of soil m. e.%	..	2 6	3 5	18 0	2 4	9 0	15 0	5 4	12 5	31 0
1 Total nitrogen of soil saturated with NH_4	Replaceable m. e.%	11 0	31 0	101 0	11 6	32 5	97 0	17 6	32 5	117 0
	Exchange capacity for NH_4 m. e. %	8 4	25 5	83 0	9 2	22 3	82 0	12 2	21 0	86 0
2 Dist. with MgO	Replaceabl m %	7 7	24 5	73 0	8 8	22 7	70 0	11 8	20 4	79 5
	Fixed — m. e. %	0 7	1 0	10 0	0 4	0 8	6 0	0 4	0 6	6 5
	Fixed — % of total	5 0	3 9	12 0	4 4	3 3	7 3	3 3	2 5	7 5
3 Dist. with N NaOH	Replaceable m e. %	8 0	24 5	77 0	8 9	23 0	74 0	11 4	20 3	77 0
	Fixed — m. %	0 4	1 0	6 0	0 3	0 5	8 0	0 8	0 7	9 0
	Fixed — % of total	3 0	3 9	7 0	3 5	2 2	9 6	6 6	3 1	10 4
4 Dist. with N KOH	Replaceable m. %	7 9	24 2	73 0	8 6	22 2	71 5	11 5	20 2	76 0
	Fixed — m. e. %	0 3	1 3	10 0	0 6	0 7	10 5	0 7	0 8	10 0
	Fixed — % of total	6 2	4 0	12 0	6 6	2 9	12 6	5 8	5 8	11 6

(*Continued on Page 105*)

TABLE 2 (*Contd.*)

Ammonium fixation in the various fractions of the soil

(Taking exchange capacity of the soil on digestion of the soil for total nitrogen)

Method of replacing ammonium from soil	Nature of the absorbed ammonium	Usar (0-6")			Uncultivated (0-6")			Cultivated (0-6")		
		Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
5 Dist. with $Ba(OH)_2$	Replaceable m. e. %	7 8	24 6	75 0	8 7	22 7	75 0	11 7	20 5	97 0
	Fixed — m. e. %	0 6	0 9	8 0	0 5	0 8	7 0	0 5	0 7	7 0
	Fixed — % of total	7 5	3 5	9 8	5 5	3 4	8 4	4 1	3 3	8 1
6 Dist. with $Ca(OH)_2$	Replaceable m. e. %	7 5	24 7	73 0	8 8	22 0	73 0	11 6	20 4	76 0
	Fixed — m. e. %	0 8	0 8	10 0	0 5	1 5	9 0	0 6	0 6	10 0
	Fixed — % of total	8 1	3 1	12 0	4 4	6 3	10 8	4 9	2 8	11 0
7 Leaching with $BaCl_2$	Replaceable m. e. %	7 7	21 0	74 0	8 6	21 0	74 0	10 8	18 7	72 0
	Fixed — m. e. %	0 7	1 5	9 0	0 0	2 5	8 0	1 4	2 3	14 0
	Fixed — % of total	8 4	5 8	10 8	6 0	8 1	9 6	11 0	10 8	16 2

TABLE 3

Contribution of different fractions of soil in ammonium fixation

(Taking exchange capacity of the soil on digestion of the soil for total nitrogen)

Soil	Fraction	% of Fraction	Contribution of different fractions on: Dist. with MgO m. %	Dist. with N NaOH m. %	Dist. with N KOH m. %	Dist. with $Ba(OH)_2$ m. %	Dist. with $Ca(OH)_2$ m. %	Leaching with N $BaCl_2$ m. e. %
1 Usar (0—6")	Sand	67 00	0 469	0 268	0 335	0 402	0 536	0 469
	Silt	23 00	0 230	0 230	0 209	0 207	0 184	0 345
	Clay	10 58	1 058	0 635	1 058	0 846	1 058	0 952
	Total	100 58	1 757	1 133	1 692	1 455	1 778	1 766
	Soil		1 700	1 300	1 800	1 400	1 600	1 500
2 Uncultivated (0—6")	Sand	54 83	0 219	0 164	0 329	0 274	0 274	0 329
	Silt	21 00	0 168	0 105	0 147	0 168	0 315	0 525
	Clay	25 00	1 500	2 000	2 605	1 750	2 250	2 000
	Total	100 83	1 887	2 269	3 101	2 192	2 839	2 854
	Soil		2 100	2 200	2 600	2 300	2 700	2 200
3 Cultivated (0—6")	Sand	65 00	0 260	0 520	0 455	0 325	0 390	0 910
	Silt	20 08	0 120	0 141	0 161	0 141	0 120	0 402
	Clay	15 08	0 980	1 357	2 508	1 056	1 508	2 111
	Total	100 16	1 360	2 018	2 124	1 522	1 918	3 483
	Soil		1 400	2 100	2 300	1 600	2 100	3 600

for interlayer cations like Ca^{++} Mg^{++} Na^{+} and H^{+} in the expanded lattice of clay minerals Further the replacement of interlayer cations by ammonium results in contraction of the lattice and thus the trapping of the ammonium ions in it Another mechanism of ammonium adsorption by H saturated clay in which NH_4^+ ions and H^+ -clay react to form NH_4^+-clay was suggested by Cornet (1943) Buswell and Dudenbostel (1941) Forges (1929) put forth his theory of ammonium-organic-complex, when the soils are leached with ammonium salts and thus cause the ammonium fixation

In this study applications of the proposed method for the determination of ammonium fixing capacity was illustrated by the difference in the exchange capacity of the soil and the amount of NH_4 replaced from an ammonium saturated soil by distillation with MgO in case of normal soil and with NaOH in case of Usar soil As the whole of NH_4^+ from an NH_4 saturated soil is replaced by any of the bases studied in determining the exchange capacity of the soil so the exchange capacity of the soil, was estimated by subtracting the total N of the soil from the total N of the soil found after saturating soil with NH_4 and expressing the value as m.e.%.

Summary

Ammonium fixing capacity of Usar cultivated and uncultivated soils was tested using different bases as distillant. It was observed that in normal soils MgO distillation gave out the maximum amount of replaceable NH_4 whereas in Usar soil NaOH distillation showed better result. Ba distillation is more effective than leaching by Ba in replacing adsorbed NH_4 Clay fraction of the soil fixed highest amount of NH_4

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for interlayer cations like Ca^{++} Mg^{++} Na^{+} and H^{+} in the expanded lattice of clay minerals. Further the replacement of interlayer cations by ammonium results in contraction of the lattice and thus the trapping of the ammonium ions in it. Another mechanism of ammonium adsorption by H saturated clay in which NH_4^+ ions and H^+ -clay react to form NH_4^+-clay was [illegible] by Cornet (1943) Burwell and Dudenbostel (1941) Porges (1929) put forth his theory of ammonium-organic-complex, when the soils are leached with ammonium salts and thus cause the ammonium fixation

In this study applications of the proposed method for the determination of ammonium fixing capacity was illustrated by the difference in the exchange capacity of the soil and the amount of NH_4 replaced from an ammonium saturated soil by distillation with MgO in case of normal soil and with NaOH in case of Usar soil As the whole of NH_4^+ from an NH_4 saturated soil is [illegible] replaced by any of the bases studied in determining the exchange capacity of the soil so the exchange capacity of the soil was estimated by subtracting the total N of the soil from the total N of the soil found after saturating the soil with NH_4 and expressing the value as m e.%

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Ammonium fixing capacity of Usar cultivated and uncultivated soils was tested using different bases as distillant It was observed that in normal soils MgO distillation gave out the maximum amount of replaceable NH_4 whereas in Usar soil NaOH distillation showed better result. Ba distillation is more effective than leaching by Ba in replacing adsorbed NH_4 Cl[illegible] tion of the soil fixed highest amount of NH_4

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AGRO ECONOMIC STUDY OF CROPPING PATTERN

S. P Dhondyal*
Agricultural College Kanpur

Introduction

Broadly speaking, the term cropping pattern refers to the distribution of crops crop combinations and rotation programmes followed over a period on individual farm units or on the aggregate area of a village, district, State or the country as a whole The type of cropping pattern followed by a cultivator is not the result of his whims or fancy but is the outcome of his long experience and is adapted to the economic opportunities of that area. In many instances personal preference, traditions and social considerations affect the cropping patterns of individuals.

As we view the over-all picture of the cropping pattern in India, we find that great variations in climate rainfall soil and topography have provided the country with almost every kind of food and agricultural raw produce The geographical distribution of crops in India may in a general way be described as wheat in the northern region rice in the eastern part, fruits in the Western Himalayas tea in the eastern Himalayas, and millets in the southern region.

No doubt natural factors like soil and climate specify the range of crops which can be grown in a locality but economic and technical factors are extremely important. Irrigation facilities cost of production per acre seasonal distribution of labour capital needs, accessibility to market personal preferences of the cultivators, introduction of new crops and high yielding varieties problems of soil conservation and drainage enterprise relationships and livestock systems together with the relative prices for different crops, are factors which help to determine which crops or cropping pattern on individual farm units or groups of farms will be most profitable.

Objects and Scope of the Study

The main purpose of the present inquiry is to ascertain the changes in cropping at a *macro-level* and work out the economics of cropping patterns at the *micro* or *individual farm level* for different size holdings.

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*Head of the Deptt. of Agri. Econ., Govt. Agri. College Kanpur

Macro-Level change in Cropping Pattern

From the standpoint of agricultural production in India, the cultivated area was increased from 2 98,618 thousand acres, the annual average of the three years 1949 50 to 1951 52 to 3 43 426 thousand acres for the annual average of the three years 1958-59 to 1960-61 With the increase in the cultivated area there has been a slight change in the cropping pattern as will be seen from the following table 1

TABLE 1

*Percentage distribution of the cultivated area under different crops and the yield per acre **

Crop	% distribution		Difference	[illegible]
	1951 52	1960-61		
Rice	25 15	24 03	−1 12	[illegible]
Jowar	13 23	12 31	−0 91	[illegible]
Bajra	8 55	8 01	−0 51	[illegible]
Barley	2 62	2 38	−0.24	[illegible]
Wheat	8 06	9 26	+1 20	[illegible]
Cotton	4 96	5 60	+0 61	[illegible]
Groundnut	4 00	4 36	+0 36	[illegible]
Gram	6 30	7 16	+0 86	[illegible]
Other crops	27 11	26 86	−0 25	

The change in relative proportions of crops indicates that some [illegible] is perceptible in the cropping pattern in the direction of an increase in [illegible] of those crops which give relatively high yield per acre except [illegible] case of rice The area of rice increased from 75 106 thousand [illegible] 82,534 thousand acres during the period under review even though it [illegible] decrease percentagewise.

*Source—Ind. Jour of Agri. Eco. Vol. XII No. 1 (1962).

TABLE 2

*Percentage distribution of the cropped area by different crops and crops yield per acre in U P **

Crop	Percentage distribution		Difference	Average yield per acre in Mds. 1958–59 to 1960–61
	1951 52	1960-61		
Rice	20 00	19 90	−0 10	7 27
Jowar	4 88	4 25	−0 63	6.85
Bajra	5 42	5 18	−0 24	5 59
Maize	4 32	5 01	+0 69	7 63
Wheat	17 21	18 73	+1 52	9 59
Barley	10 11	8 78	−1 33	9 02
Gram	12 66	12 14	−0 52	6 99
Sugarcane	5 26	6 32	+1 06	349.24
Cotton	0 22	0 30	+0 08	1 31
Other crops	19.92	19 39	−0 53	

There has been some change in the cropping pattern in U P where the percentage distribution of total cultivated areas has increased under sugarcane, maize, wheat and cotton and decreased under gram rice jowar bajra and barley The change is not related to any cropping system based on per acre yields but to the relative net incomes from different crops. The area under sugarcane cultivation has considerably increased from 25 04 lakh acres in 1951 52 to 32·83 acres in 1960 61 because of the profitable production of the crop as the result of a guaranteed minimum price fixed by the Government.

With the expansion of irrigation facilities and technical knowledge the intensity of cropping is increasing in as much as the area sown more than once has increased from 90 18 lakh acres in 1950-51 to 110 28 lakh acres in 1960-61

Micro or farm Management Level :

(1) COST ACCOUNTING METHOD

Case Study Farms—The economics of cropping pattern was worked out on four cultivators holdings on the basis of data collected by the Agricultural Economics Section of this College through cost accounting method. The

* U P Agric. Statis. Bulletin

figures presented give some idea of the influence of urbanization and characteristics of cropping patterns.

FARM No. 1

The study is related to one cultivator's holding in the village ..., situated at a distance of 1½ miles from Kanpur city. The area of the holding is 6.5 acres. The number of family workers are 3. The percentage of area under irrigation is 41.

Owing to the proximity to the city the cropping pattern is ... with specialization in vegetable raising with a high degree of intensity of cropping being 192.54% for the year 1960-61 (Table 3, p. 5)

TABLE 4

Percentage Distribution of Crops sown

Sl. No.	Crops	1959-60	1960-61
1	Cereals	33 01	31 77
	Vegetable	44.38	47 56
3	Pulses ...	8 91	7.60
4	Millets	6 87	2.57
5	Fodder	6 77	7 49
		100 00	100 00

PERCENTAGE DISTRIBUTION OF CROPS SOWN

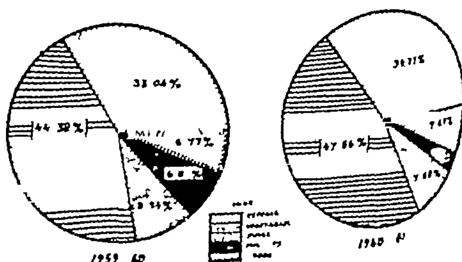


TABLE 3

Cropping Scheme

Sl. No.	Acres in plot	1959-60 Crops			Total acres of crops	Acres in plot	1960-61 Crops			Total area of crops
		Kharif	Rabi	Zaid			Kharif	Rabi	Zaid	
1	0 56	Paddy	--	--	0 56	0 62	Paddy		Toral	1 24
2	1 44	Toral	Wheat		2 88	0 31	Jowar +	Arhar	--	0 31
3	0 72	Khira	Potato	Musk M lon	2 16	0 40	Chari		Toral	0 80
4	1 03	F llow	Wheat		1 05	0 72	Khira	Potato	Pumpkin	2 16
5	0 09	Chari	Potato		0 18	1 44	Khira	Wheat	--	2 88
6	0 31	Chari	Pea		0 62	1 03	Toral	Barley	Pea	2 06
7	0 22	Chari			0 22	0 09	Chari	Potato	--	0 18
8	0 63	Jowar +	Arhar		0 63	0 31	Chari	Barley		0 62
9	0 38	Tomato			0 38	0 84	F llow	Wheat		0 84
10	0 19	Fallow	Pea		0 19					
Total 5 57					8 85	5 76				11 09
Intensity of cropping					159 00					192 54

The cultivator has organised his cropping plan to increase the production of high profitable crops. Location transportation and accessibility to market gave him an advantage over any other area for producing vegetables.

The following figures give the financial position of his farm business

TABLE 5

Per acre (in rupees)

Sl. No.	Particulars	Years	
		1959-60	1960-61
1	Capital investment	223 35	213 19
2	Net farm income	168 90	196.29
3	Family labour income	170 68	2.7 75
4	Farm business income	193 72	210 13

The farm economy is representing a cropping pattern in which about half of the area is under vegetables, thereby giving an average net farm income of Rs 183 00 per acre

FARM NOS. 2 3 AND 4

The study is related to the three farms of the village, Bari Ah... situated at a distance of about three and a half miles from Karna... The percentage of irrigated area is 16 5 % only The size of holdings of the farmers Nos. 1 2 and 3 is 10.51 8 82 and 7.24 acres respectively ... respective numbers of actual family workers converted into adults ... and 2

Due to the absence of proper irrigation facilities, the ... cropping on an average is about 112 only The crop land ... fallow during the kharif season partly because about 30 of the ... *Kachhar* is submerged under water during the rainy season ... added to lowering the cropping intensity

TABLE 6

Cropping Scheme

Sl. N	Farm No. 1			Farm N 2			Farm No 3		
	Plot size in acres	Crops Kharif	Crops Rabi	Plot size in acres	Crops Kharif	Crops Rabi	Plot size in acres	Crops Kharif	Crops Rabi
1	1 75	Jowar+urd+Arhar		0 63	Moong –	Bejhar	0 62	Fallow –	Gujal
2	1 50	Fallow – Wheat+Barley		0 30	Charl –	Bejha	0 62	Maize +	Arhar
3	0 40	Fallow –	Wheat	0 94	Jowar +	Arhar	1 25	Jowar +	Arhar
4	0 75	" –	Bejhar	2 83	Fallow –	Wheat	0 33	Fallow –	Wheat
5	3 00	–	Barley	0 30	Maize +	Arhar	0 87	Fallow –	Gujal
6	0 57	" –	Barley	0 78	Jowar –	Bejhar	0 25	Urd –	Gujal
7	1 00	Sugarcane (Ratoon)		0 50	Sugarcane		0 62	Fallow –	Carrot
8	0 57	Urd+Jowar+Arhar		0 50	Paddy –	Bejhar	1 58	Fallow –	Gujal
9	1 37	Fallow –	Barley	0 78	–	Bejhar	1 00	Fallow –	Bejhar
10				0 63	–	Hariari			
11				0 63	Paddy	Chatari			
Total	10 31			8 82			7 14		
Intensity of Cropping		100 00%			132 20%			103 50%	

The nature of cropping pattern shows that the sugarcane is relatively more important on small holdings below 5 acres and on larger holdings above 20 acres Wheat is another important crop which has relatively higher percentage of acreage on holdings about 30 acres. Cotton has a higher percentage on holdings between 15 and 20 acres, but cultivators of all the holdings operate relatively smaller acreage because of its lower value per acre.

The figures presented below give some idea of the financial returns obtained from the two most important crops of the area around which main rotations have been established.

TABLE II

Cost and Returns per Acre

Crops	Size groups in acres	Total Input	Output in Mds.	Value of output in Rs	Net Profit in Rs	Cost of production per maund in Rs
1 *Sugarcane*	Below 5	400	594	568	168	[illegible]
	5 to 10	347	402	578	231	[illegible]
	10 to 15	308	406	581	276	[illegible]
	15 to 20	324	415	596	27?	[illegible]
	20 to 30	255	363	521	?66	[illegible]
	30 and above	247	340	493	246	[illegible]
	Average	314	387	557	15	[illegible]
2 *Wheat*						
	Below 5	265	13.5	255	—18	[illegible]
	5—10	221	12 8	239	—18	[illegible]
	10—15	165	12 2	230	65	[illegible]
	15—20	162	11 3	213	53	[illegible]
	20—30	154	11 1	209	55	[illegible]
	30 and above	136	11 0	191	56	[illegible]
	Average	200	12 0	223	23	[illegible]

Note: The cost of production per maund of wheat was arrived at [illegible] proportion to the relative incomes obtained for wheat grains and [illegible]

The cost of cultivation per acre of sugarcane decreased in [illegible] an increase in the size of holding being Rs. 400/ on holdings below 5 acres and Rs 247/- on holdings 30 acres and above The net profit per acre

was highest on holdings between 10 and 15 acres. The cost of production per maund was lowest on holdings between 20 and 30 acres In the case of wheat the cost of cultivation and net profit per acre showed the same tendency as in the case of sugarcane. The cost of production per maund was lowest on holdings 30 acres and above.

Technical Factors—The analysis detailed above relates to the cropping pattern as determined by natural and economic factors. There is another consideration namely the technical factor which also contributes to the drawing up of a cropping programme for a farm.

Better management practices and tillage methods introduction of a new crop use of high yielding crop varieties effectiveness of insect and disease control and the like when tied together bring about changes in cropping programme in term of the choice of crops and acreage under each crop and rotations to be followed.

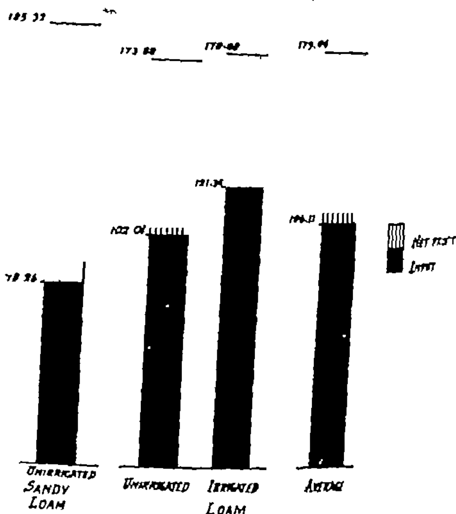
One of the great revolutions in agriculture has been the evolvement of high yielding varieties and introduction of new crops Groundnut occupied a very small proportion in the crop rotation programme in U P till 1926 when its area was about 13 000 acres only but gradually with the introduction of high yielding strains the acreage under the crop went up to 4 78 lakhs in 1959-60 The districts of Etah, Bareilly Badaun Moradabad Farrukhabad Sitapur and Hardoi are noted for its important place in the cropping scheme of the cultivators who are adopting the improved two year rotation of groundnut+arhar—sugarcane

A study of the economics of groundnut cultivation was conducted on 75 cultivators holdings in Hardoi district in 1960-61 The results are summarised as below —

TABLE 12

Costs and Returns per Acre

Types of soil	Total output in mds.	In Rupees			Cost of production per md in Rs
		Input	Value of output	Net Profit	
Sandy loam					
Unirrigated	13 23	78 26	185 32	107 06	5 91
Loam					
Unirrigated	12 42	102 01	173 88	71 87	8 21
Irrigated	12 72	121 35	178 08	56 73	9 54
Average of all holdings	12 79	106 11	179 06	77 95	8 30



Sandy loam soil is best suited to groundnut cultivation giving a [illegible] profit of Rs. 107 06 per acre as against the irrigated loam soil which gives [illegible] net profit of Rs 56 73 per acre.

Another example is of the progressive development of sugarcane [illegible] in U P and other parts of India. Sugarcane yields from the old [illegible] were very low and hence its area was very limited. But with the [illegible] tion of new high yielding strains sugarcane has occupied an importa[nt] [illegible] in the cropping scheme all over India where soil and climatic factors [illegible] its cultivation Thus sugarcane wheat-cotton in the Punjab, sugarcane [illegible] or paddy in U P sugarcane paddy in Bihar sugarcane-sugarcane [illegible] wheat or gram in Bombay and sugarcane-paddy in Madras are [illegible] sugarcane rotations affecting the organisation or plan of the farm.

In U P the recent introduction of Moong T_1 a quick growing crop of about 65 days duration is tending to introduce a change in the cropping system from fallow wheat to Moong T_1-wheat as a definite contribution to increased productivity in the state and at the same time maintaining the fertility status of the soil.

Summary and Conclusions

The cropping pattern followed in different geographical regions of the country is the result of the long experience of the cultivators to devote their land labour and capital to the production of those crops which are best suited to the natural factors namely climate topography and soil of the area. These factors determine the yield per acre and hence the cost of production per quintal, but they do not specify which crops crop combinations and rotations are most profitable. Hence economic factors affecting prices received by the cultivators for their produce would indicate the choice of crops best suited to the locality and the acreage to be kept under each crop. Further technical factors like the introduction of high yielding crop varieties and adoption of better farm management practices also bear influence on the range of cropping pattern adopted on individual farms

The studies made herein show that the vegetable production was concentrated in the Vinayakpur village because of nearness to the Kanpur city as the consuming centre. The cultivators on an average, earned Rs 183·00 as net income per acre. But proximity to the city is not the only consideration for market vegetables. Irrigation is a very important force in determining the cropping pattern and levels of farm earnings The difference in net income as between the holdings in the Beri Akbarpur village, which is also fairly near to the city can be attributed to the irrigation facility available in the holding no 3 and the consequent adoption of vegetable growing on 7·36% of its area.

The studies in the Meerut district show that intensity of cropping increased with the decrease in the size of holdings. It was highest on the holdings below 5 acres The acreage under sugarcane was also highest on small holdings below 5 acres. It was further noted that price was the most important factor influencing the acreage under sugarcane because of the guaranteed minimum price fixed by the Government. The soil and resources of the cultivators favoured the growth of both sugarcane and cotton but the relative prices and differences in cost made sugarcane cultivation more profitable.

In the technical field, the introduction of improved rotations, namely groundnut+arhar—sugarcane and moong T_1—wheat in place of fallow-wheat has given more economic benefits to the cultivators within the framework of their resources.

With the development of irrigation and transport facilities, greater use of fertilizers, and the rapid progress in scientific agriculture particularly in the direction of the evolvement of high yielding varieties, quick growing crop strains and farm management practices, the cropping pattern is bound to change more towards intensive farming as to give greater returns to the resources of the cultivators.

FLORAL MORPHOLOGY AND EMBRYOLOGY OF *VERNONIA CINERASCENS* SCHULT AND *V CINEREA* LESS

B TIAGI AND SHEELA TADDU*
University of Jodhpur Jodhpur (India)

Embryologically the Compositae presents certain interesting features Besides some unclassified cases a number of different types of embryo sacs are known in the family The haustorial synergids are frequently present and the haustorial antipodals show variations in their number in the number of nuclei in each cell and in the reported occurrence of an 'antipodal oosphere Apospory parthenogenesis and polyembryony are known in a number of species. Nevertheless, the family shows remarkable uniformity in the development of the capitulum, florets, male gametophyte, embryo and seed

MATERIAL AND METHODS

Flowering material of *V cinerascens* was collected in 1959 during the rainy season from the outskirts of Jodhpur city It grows as a woody shrub generally protected by the clumps of a dendroid *Euphorbia* Since viable seeds are not formed in this species *V cinerea* which commonly grows in waste places was collected for the study of the fruit and seed.

Before processing, the heads were cut vertically into two and the mature florets treated individually The young heads were simply chopped at the top and sides for an easy infiltration The material was then fixed in F.A.A. and Acetic-alcohol Dehydration clearing and embedding were done by the xylol-paraffin method. Sections were cut at 10 to 15 μ thickness, depending on the age of the material. Haidenhain's Iron alum-Haematoxylin Safranin Fastgreen and Gentian violet Erythrosin were used for staining

CAPITULUM

The capitulum is surrounded by approximately five series of bracts. The two outermost whorls are each made up of three or four and the rest of five or six bracts. On the peduncle and bracts there are thick walled peltate hairs (Fig 10)

The young capitulum develops a number of rounded protuberances (Fig 1) which gradually change into tubular florets (Figs. 2-4) Pappus is belated in development. The sequence being petals stamens, pappus and the gynoecium. There are about fourteen florets in a capitulum of *V cinerascens* (Fig 4) and twentyseven in *V cinerea*.

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* Present address Lecturer Department of Education, Govt. of Rajasthan, Udaipur

With the development of irrigation and transport facilities, greater use of fertilizers and the rapid progress in scientific agriculture particularly in the direction of the evolvement of high yielding varieties, quick growing crop strains and farm management practices, the cropping pattern is bound to change more towards intensive farming as to give greater returns to the resources of the cultivators.

Ovule

The ovule is anatropous unitegmic and tenuinucellate (Figs 6-8) Venkateswarlu and Maheswari Devi (1956 b) observed in some ovaries of *Tagetes*, two ovules which sometimes showed various degrees of fusion. Mestre (1957) also found in numerous ovaries of *Centaurea collina* two ovules one of which however aborts later

The funicular vascular bundle runs upto the chalaza only (Figs. 6-8) Integumentary vascular bundles however are known in *Tagetes Flavena* (Venkateswarlu and Devi 1955 b) and *Tridax* (Maheshwari and Roy 1952)

Nucellus degenerates and the innermost layer of the integument surrounding the embryo sac forms a glandular endothelium of uninucleate cells (Fig 8) It exerts a powerful influence in the nutrition of the embryo sac Ultimately however it is simply absorbed along with the integument and some inner layers of the pericarp. Deshpande (1960 1962) believes, however that the endothelium is persistent and it stores starch

Microsporogenesis and the Male Gametophyte

There are four wall layers in the anther epidermis endothecium middle layer and the tapetum (Fig 15) The microspore mother cells are arranged in a single row in each loculus (Fig. 16) Epidermis is persistent endothecium develops meagre fibrous thickenings the middle layer and the tapetum are absorbed (Fig 18) One middle layer is usually present in many Compositae and a fibrous endothecium also is ordinarily present. Venkateswarlu and Devi (1955 a, b) and Devi (1957) however report absence of the fibrous thickenings in the endothecium.

Tapetum is of glandular type. It is uninucleate at the time of differentiation of the microsporocytes (Fig 15) Its activity however is at its optimum when the microsporocytes are preparing for meiotic divisions (Figs. 16, 17) Tapetal nuclei become spindle shaped and highly polyploid by successive divisions and fusions (Fig 17) Two such giant nuclei are usually found in a tapetal cell (cf Davis, 1961 a)

Microsporocytes secrete a mucilaginous substance around their protoplasts, inside the cell wall (Figs. 16, 19 20) The nucleus divides meiotically giving rise to a tetrad of microspores (Figs. 19-21) Quadripartition of the mother cells takes place by furrowing (Figs. 22, 23) The nucleus surrounded by dense cytoplasm is centrally located in the microspore and vacuoles appear at the periphery of its protoplast (Fig. 24) Soon afterwards however the vacuoles disappear and the pollen develops the characteristic echinate ornamentation of the exine (Fig 25) The protoplast of the two celled pollen gets drawn towards the four germ pores (Fig 26) Vegetative nucleus is rounded while the generative is rather flattened At the time of shedding pollen grains

are three celled (Fig 28) Exine possesses rounded germ pores and reticulations bearing pointed processes (Fig 27) Protoplast of the mature pollen grain reveals a peripheral region of finer and the inner of coarser cytoplasm (Fig 28) Intine usually protrudes at the germpores and it appears that the vegetative nucleus enters first in the pollen tube followed by the crescent shaped male gametes (Fig 28) Tri- and tetra-colporate echinate and three celled pollen grains appear to be a common feature of the family Banerji (1940 a b) however reported binucleate pollen grains in *Tridax* and trinucleate in *Carthamus* but these were found to be three celled as usual, by later workers.

Megasporogenesis and the Famale Gametophyte

There is a single hypodermal archesporial cell which directly functions as the megaspore mother cell (Figs 6 7 29) It enlarges considerably and divides meiotically to give rise to a dyad (Fig 30) and a linear tetrad of megaspores (Fig 31) Maheshwari and Roy (1952) found two such tetrads arranged in a linear fashion The chalazal megaspore functions and develops into the Polygonum type of embryo sac (Figs. 36-39) Devi, (1957) found that in a few cases both the micropylar and the chalazal megaspores had developed into four nucleate embryo sacs. Double and triple embryo sacs occur in *Tagetes* probably due to the development of two and three megaspores respectively (Venkateswarlu and Devi 1955 b)

The oosphere differentiates rather early (Fig 38) Pear shaped, hooked synergids (Fig 40) become conspicuously large and haustorial (Fig 41). The egg protrudes beyond the synergids into the cavity of the embryo sac (Fig [illegible] Polars seem to pair in the middle of the embryo sac but the large secondary nucleus lies near the egg apparatus

Large haustorial synergids often extending into the micropyle appear to be a common feature of the family The synergids in *Cotula australis* however extend deeply into the micropyle and one persists until late in embryogeny as a haustorium (Davis, 1962) According to her (1961 c) the transformation of a synergid into a micropylar haustorium has not been reported previously in the family Antipodals also become pear shaped and haustorial, [illegible] in the chalaza (Fig 39) They may divide further to form a [illegible] antipodal tissue consisting of uninucleate cells in *[illegible]* (Fig 43) In the Compositae the antipodals show a number of many other [illegible] ing features. Mitra (1947) noted great variability in the antipodals regarding the number of its cells and nuclei. Martin and Smith (1955) [illegible] antipodals one binucleate the other uninucleate Additional [illegible] occur in them Devi (1957) noted that the organization of a [illegible] place earlier than that of the egg apparatus There are two [illegible] dals, each with two to twelve nuclei There are two or three antipodals [illegible] *Cotula australis* (Davis, 1962) The number of nuclei may increase [illegible]

in each and these may fuse together The three antipodals in *Podolepis* may undergo active division resulting into as many as fiftyone cells. Further the antipodal region is haustoral and persists as a vermiform appendix (Davis, 1961 b) Three persistent uninucleate antipodals have been reported by Venkateswarlu and Devi (1955 a) and Berger Feeley and Witkus (1956) However increase in the number of their nuclei is a common feature. In *Eclipta erecta* (Bhargava 1935) there are three uninucleate antipodals or there may be two one binucleate and the other uninucleate. According to Banerji (1940 a) there are two or three normally binucleate but sometimes trinucleate antipodal cells in *Tridax procumbens* Maheshwari and Roy (1952) confirmed the number of antipodals as two or three, being equally common. Later they become multinucleate and frequently fuse to form a single nucleus with many nucleoli. Venkateswarlu and Devi (1955 b) report that in Heleniese, there are two to three antipodals but the number of nuclei in each cell is variable, one or two in *Flaveria* (cf. Misra, 1957) and one to six in *Tagetes*

Antipodals simulate the egg apparatus in *Gillardia* (Venkateswarlu and Devi, 1955 b) and *Rudbeckia* (Maheshwari and Srinivasan, 1944) An antipodal resembles the egg and is suggestive of an oosphere while the lateral antipodals simulate the synergids. The latter may degenerate soon after fertilization 'antipodal oosphere however is persistent. Diettert (1938) mentions an interesting case in *Artemisia tridentata* the innermost antipodal cell penetrates the chalazal tissue and may even enter the ovarian chamber

Bisporic, Allium type of embryo sac also occurs in some florets of *Vernonia cinerascens* The micropylar dyad cell degenerates while the chalazal develops further (Figs 32 33) Sometimes the micropylar dyad may divide by a vertical wall resulting in a triad (Fig 34) The two megaspores degenerate while the chalazal dyad is functional In a dyad, the chalazal cell developed into a two nucleate embryo sac while the micropylar is persisting (Fig 35)

Allium and Polygonum types of embryo sacs occur in *Tridax trilobata* (Hjelmqvist, 1951) Banerji (1940) and Maheshwari and Roy (1952) found however only the latter type in *Tridax procumbens* Avanzi (1948) reported bisporic embryo sac in *Ammobium alatum*. The chalazal dyad develops further The nucleus of the upper dyad cell, however may undergo one division before degeneration. Harling (1951) noted bisporic development in five species of *Chrysanthemum* and also found pseudo-bisporic embryo sacs (*see* Maheshwari, S.C. 1955) Embryo sac is of Polygonum type in *Leontodon hispidus* but in many cases all the megaspore nuclei divide to form a tetrasporic, Adoxa type of embryo sac (Bergman 1935) Maheshwari and Haque (1949) found a tetrasporic embryo sac in *Chrysanthemum parthenium* having variable number of nuclei and showing variability in the organization of the antipodal cells Fritillaria type of embryo sac has been reported by Maheshwari and Srinivasan (1944) and Venkateswarlu and Devi (1955 b) Urbanska (1956) described aposporic

embryo sacs in *Homogyne alpina*. Embryo sac development is known to be of an aberrant type in some cases (*see* Maheshwari, 1950)

Fertilization

Entry of the pollen tube is *porogamous*. *Double fertilization is a normal* [illegible] taneous process Banerji (1940 b) noted in *Carthamus* that the integumentary cells lining the micropyle develop into long hairs which are directed to [illegible] the egg apparatus probably facilitating the entry of the pollen tube into the embryo sac. Synergids disintegrate but the remnants of one synergid and the pollen tube are persistent (Figs 44 45) Persistent pollen tubes have al[illegible] been reported in this family by Venkateswarlu and Devi (1955 a, b) and [illegible] possesses an apical pore in *Launaea pinnatifida*

Endosperm

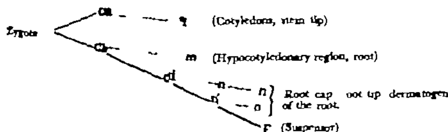
Endosperm is cellular A number of cells accumulate before the division of the zygote The first division of the primary endosperm nucleus is trans- verse resulting in a micropylar and a chalazal chamber (Fig 41) Although endosperm develops in both it is the micropylar chamber where greater activity occurs and earlier too The second division which is vertical [illegible] some more divisions also take place in the micropylar chamber the chalazal remaining uninucleate in the m antime (Fig 45) The endosperm cells are large, uninucleate and vacuolated (Fig 46) Vacuoles, however gradually disappear as the cytoplasm is filled densely with starchy *food material*. Desh- pande (1960 1962) found that the endosperm cells in *Carduus* and *Tridax* are poorly cytoplasmic and highly vacuolated. *It is in this condition that they* are completely absorbed by the embryo

Endosperm in the Compositae is generally Cellular when Nuclear *it* [illegible] Cellular rather early Orientation of the first wall when Cellular is [illegible] constant Dahlgren (1920) noted that this may be *transverse* [illegible] even oblique in a genus or even for a species. *In Eclipta erecta* ([illegible] 1935) the endosperm is Cellular and the first wall is transverse Venka- teswarlu and Devi (1955 a) reported that the endosperm is Cellular in *Launaea* but in Helenieae (1955 b) it is Nuclear Endosperm in [illegible] to be Cellula (Berger et al 1956) and sometimes the division of the [illegible] endosperm nucleus is delayed. They observed a spherical embryo in one case although the endosperm nucleus *was still undivided* Small [illegible] (1917) observed that the primary endosperm nucleus may remain [illegible] although the embryo becomes many celled Endosperm is Cellular in [illegible] and *Melanthera* but it is *partly Cellular* and *partly Nuclear in* [illegible] It is Nuclear in *Carthamus* Banerji 1940 b In *Tridax* endosperm is [illegible] (Maheshwari and Roy 1952) but wall formation takes place *very* [illegible] few cases a proembryo existed along with an undivided primary [illegible] nucleus. Endosperm is Cellular in *Cotula australis* (Davis 1[illegible]) One [illegible] sac contained a proembryo and the primary endosperm nucle[illegible]

the author infers that the embryo is not dependent on the endosperm in its early life.

EMBRYOGENY

Zygote elongates considerably and divides transversely into a terminal cell, *Ca*, and a basal cell *Cb* (Figs. 44 45 47). The former divides vertically into two juxtaposed cells *q* (Fig 48) and the latter divides transversely into two superposed cells *m* and *Ci*. Proembryo therefore becomes T-shaped. The cell, *m* divides vertically (Figs. 50 51) and *Ci* transversely into two superposed cells *n* and *n'* (Fig 49). Sometimes the derivatives of *q* and *m*, may become precociously bicelled each (Fig 50). An Asterad type of embryo is developed in which *q* forms the quadrant *m* divides vertically *n* becomes two celled both *O* and *p* the derivatives of *n* remain single celled (Fig 51). The nuclei of the quadrant divide again resulting into oblique segments—the octant (Fig 52). Out of the four segments of the quadrant, two may divide yielding an unusual octant consisting of six cells only (Fig 53). At the preceding stage also a segment of the quadrant had remained undivided, thereby forming a seven celled unusual octant region of the embryo (Fig 52). The *m*, and *n*, become four celled each and *n* may divide belatedly into *O* and *p* (Fig 53). Dermatogen differentiates in the segments of *q* and *m*. *O* and *p* remain ordinarily undivided but may divide transversely into two cells each (Fig 54). Globular embryo (Fig 55) becomes heart shaped (Fig 56) and later dicotyledonous (Fig 57). Suspensor is derived from the cell *p*. It normally remains as an elongated attenuated cell (Fig 55) or may become vesicular (Fig 56). Suspensor disappears in the mature embryo. A graphic account of the embryogeny is as follows



Bhargava (1935) noted that after the three celled filamentous embryo is formed the terminal cell divides by a vertical wall. By further divisions the lower region of the embryo becomes globular and a dermatogen is marked out. Mitra (1947) reported three celled suspensor in *Mikania* and *Ageratum* five celled in *Grangea*. Maheshwari and Roy (1952) concluded that the embryogeny in *Tridax* follows the *Lactuca sativa* type. In one case, they found two embryos one of them was the adventive embryo with a suspensor. Venkateswarlu and Devi (1955 a, b) and Dev (1957) described Asterad type, *Senecio* variation of embryogeny. From the descriptions of Berger et al (1956) it appears that the embryogeny in *Xanthium* is of the Asterad type. Banerji (1940 b) reported *Crepidia* type of embryo in *Carthamus* suspensor is four to eight celled. Em-

bryogeny conforms to Asterad type. In *Cotula australis* suspensor is two celled (Davis 1962)

SEED AND FRUIT

In *Vernonia cinerascens* seed formation has not been observed. Pericarp however develops normally the characteristic pappus is present. Seeds develop in *V. cinerea* (Fig 57) Embryo is straight with a long hypocotyl and the two cotyledons remain adpressed together Between the cotyledons is an inconspicuous plumule. The massive integument is almost completely liquidated and absorbed by the endosperm which in turn itself is consumed by the embryo. Only a thin layer of the endosperm persists around the embryo (Fig. 57). Embryo therefore, enveloped by this thin endosperm lies protected in the pericarp. The pericarp does not show any anatomical differentiation. Its outermost layer however becomes thick walled while the inner elongated parenchymatous layers, including the endothelium are absorbed Only its crushed remnants are left in close contact with the outermost layer of the pericarp (Fig 57)

Anatomically pericarp is well differentiated in *Galactites tomentosa* (Fourment and Rouret, 1957) Maheshwari and Roy (1952) also showed some anatomical differentiation in the pericarp of *Tridax* Closely adhering to the pericarp of *Tridax* is the testa composed of elongated cells

Seeds of Compositae are usually described as nonendospermic by the systematists Venkateswarlu and Devi (1955 a b) and Devi (1957) also state that the seed is exalbuminous and the seed coat is completely crushed by the embryo except a thin membrane consisting of one or two outer layers of the integument. Deshpande (1960 1962) believes that the seeds of *Carthamus* and *Tridax* are nonendospermic but it is *the endothelium which persists, stores* food and simulates the endosperm. Maheshwari and Srinivasan (1944) on the other hand reported one or two layers of endosperm in *Rudbeckia* [illegible] and *Tridax* (Maheshwari and Roy 1952) Fourment and Rouret (1957) found in *Galactites* that the seed largely consists of two cotyledons with a compressed almost non-existent endosperm. The embryo is enclosed in two layers of endosperm in *Cotula australis* (Davis 1962) and one layer in *[illegible]* (Davis 1961 b)

DISCUSSION

Pappus Small (1919) has emphasized that the pappus is trichome in its morphological nature. Nevertheless there appears to be some uncertainty regarding the phyllome or trichome nature of the pappus. Rao and Sayeeduddin (1958) have shown that the pappus is derived from the [illegible] primordia in *Tridax procumbens* Many other authors similarly regard the pappus as sepaline in nature (Banerji 1940 a b 1947 Venkateswarlu and Devi, 1955 b Devi 1957) One of the simplest and probably the most primitive type of pappus is found in *Vernonia*. It is just a bunch of [illegible]

the free ends of the constituent trichomes are drawn out as serrations. We are therefore, in agreement with Small (1919) and believe that the sepals have disappeared once for ever in the Compositae. Other types of pappus seen within the family can be easily derived from this type by fusion and reduction.

Ovary Subramanyam (1951) believes that probably the ovary of the Compositae has been derived from the Stylidiaceae either by suppression of the septum or abortion of one of the loculus. In this connection the occurrence of two ovules in some ovaries of *Tagetes* (Venkateswarlu and Devi, 1955 b) is significant. The ovules sometimes show various degrees of fusion. Mestre (1957) also found that numerous ovaries of *Centaurea collina* possess two ovules, one of which later aborts. The author suggests that it is a vestigial feature It may therefore be surmised that the ancestors of the Compositae were having gynoecia with two ovules in probably two separate loculi.

Endothelium Deshpande (1960 1962) mentions that it is the endothelium which stores the food material It persists and simulates endosperm while the endosperm as such is lacking This would really be very interesting if it were true Nutritive and later protective functions of the endothelium are well known but as far as we are aware a storage function has not been ascribed to it (see Tiagi and Taimni 1960)

Endothecium A fibrous endothecium is present as a rule in most of the angiosperms. Its presence however has consistently been denied for some members of the family (Venkateswarlu and Devi, 1955 a b Devi 1957) The fibrous thickenings however are conspicuously present in the endothecium of *Flaveria*—a Helineae (Misra 1957)

Seed Seeds of Compositae have been described by systematists as non-endospermic. This is probably due to its meagre occurrence which is easily overlooked by them. A feeble endosperm undoubtedly exists in *Vernonia cinerea* surrounding the straight embryo which fills the cavity of the ovary Maheshwari and Srinivasan (1944) demonstrated endosperm consisting of one or two layers in *Rudbeckia* and *Tridax* (Maheshwari and Roy 1952) Fourment and Roux (1937) described a compressed almost non-existent endosperm in the seeds of *Galactites*

In *V. cinerea* the integument is almost completely consumed by the endosperm. The embryo therefore enveloped in the thin endosperm lies naked in the pericarp Further we are of the opinion that in some cases at least the endosperm has probably been mistaken for testa (cf. Venkateswarlu and Devi 1955 a b Devi 1957) and the inner crushed layers of the pericarp for the integument (cf Davis 1961 b 1962)

SUMMARY

Floral morphology and embryology of *Vernonia cinerascens* and *V. cinerea* has been investigated. The florets are all tubular

There are five epipetalous coherent apically appendaged and tailed anthers. Tapetum is of the glandular type with giant polyploid nuclei. Microsporocytes are arranged in a single row in each loculus. Quadripartition takes place by furrowing mode of division is simultaneous. Microspores occur in tetrahedral tetrads. Pollen grains are echinate tetracolporate and three celled.

Ovule is anatropous unitegmic and tenuinucellate. Funicular vascular bundle runs upto the chalaza only. Endothelium is present. The single hypodermal archesporial cell functions directly as the megaspore mother cell. Embryo sac is of the Polygonum type also Allium type in I. *cuneatum*. Synergids are enlongated pearshaped, hooked and haustorial. Antipodals may form a multicellular tissue consisting of uninucleate cells. Fertilization is porogamous. Pollen tube is persistent. Double fertilization is simultaneous. Endosperm is Cellular. Endosperm haustoria are absent. Embryo is of the Asterad type. Straight dicotyledonous embryo enveloped in a thin layer of endosperm lies naked without a testa in the ovarian cavity. *Seeds are not set in I. cuneatum*

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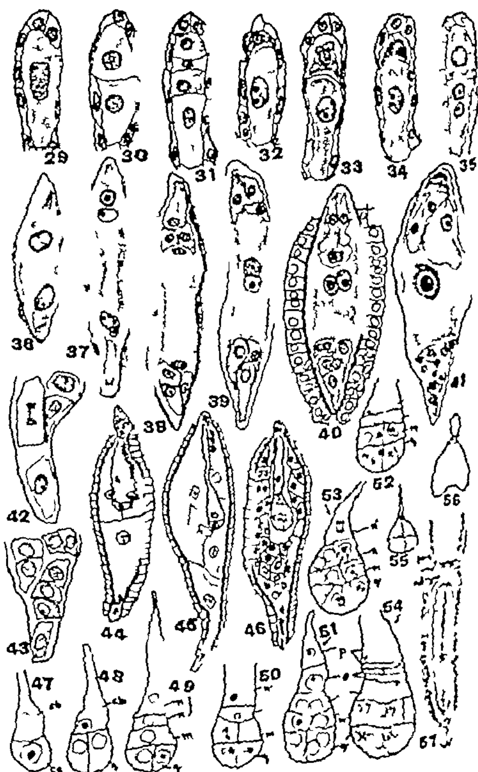
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Figs. 1-28. *Vernonia cinerascens* Fig. 1. L. s. capitulum, floral primordia. Fig. 2. L. s. young floret. Figs. 3-4. L.s. & T.s. capitulum florets. Fig. 5. L.s. a floret. Figs. 6-8. L. s. ovules. Fig. 9. a biseriate trichome and glandular hair. Fig. 10. a peltate hair. Fig. 11. stigmatic hair. Fig. 12. pappus a part magnified. Fig. 13. a gland from corolla. Fig. 14. T.s. flower bud, corolla, six stamens and stigmas. Figs. 15-16. L.s. a part of anther wall layers and microsporocytes. Fig. 17. tapetal cells, tangential view. Fig. 18. T.s. anther. Figs. 19-20. microsporocytes, dividing. Figs. 21-23. microspore tetrads. Figs. 24,25. pollen grains, uninucleate. Fig. 26. same bicelled. Fig. 27. same ornamentation of exine and germ pore. Fig. 28. same three celled. (g=gland; ne=nectary; no=notch in ovary wall; pa=pappus; =cushion shaped ring)


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Figs. 29-57 (Figs. 29-43 *Vernonia cinerascens*, the rest of *V. cinerea*). Fig. 29. megaspore mother cell. Fig. 30. dyad. Fig. 31. tetrad, megaspores. Figs. 32, 33. dyad, chalazal functional. Fig. 34. triad, megaspores degenerating, chalazal dyad functional. Figs. 35. dyad, lower cell binucleate. Fig. 36. embryo sac, two nucleate. Fig. 37. same four nucleate. Fig. 38, 39. same, eight nucleate. Fig. 40. same, initiation of antipodal tissue, polars fusing, synergids hooked. Fig. 41. same, antipodal tissue. Figs. 42-43. same, chalazal part, antipodal tissue. Fig. 44. embryo sac, micropylar and chalazal chambers, former divided vertically, proembryo two celled. Fig. 45. same, further division in endosperm; chalazal chamber uninucleate. Fig. 46. same, endosperm, globular embryo. Fig. 47. embryo, two celled. Fig. 48. same, vertical division in terminal cell. Fig. 49. same, basal cell divided into three cells. Fig. 50. same, quadrant, m four celled. Fig. 51. same, quadrant, both m & ci bicelled. Fig. 52. same, octant, oblique walls. Fig. 53. same, octant, derivatives of basal cell adding to embryo dividing. Fig. 54. same, dermatogen in q and m. Fig. 55. same, globular, suspensor tapering. Fig. 56. same, heart shaped; suspensor vesicular. Fig. 57. L. s. seed, straight embryo enveloped in meagre endosperm, enclosed in pericarp. (end = endosperm, pa = pappus, r = cushion shaped ring).

FLORA OF MEERUT COLLEGE CAMPUS*

V Singh
School of Plant Morphology Meerut.

The Meerut College campus occupying an area of about 100 acres, lies at 29° 01 N latitude and 77° 45 E longitude, and has an elevation of 730 feet above sea level The soil is chiefly sandy loam

The average annual rainfall of Meerut is nearly 30 inches the maximum being in the months of July to September The temperature shows great fluctuations in summer and winter The minimum temperature goes down to 2° C in cold frosty nights of January and the maximum rises up to 45° C in hot noons of June. Chills are common in winter dawns while summer is characterised by strong hot winds in noons. As a whole the climate is arid.

The wild herbaceous flora is at its zenith during the rainy season because of high humidity and moderate temperature, both favourable for plant growth. With the first showers of the monsoon seeds of most of the herbaceous plants which remain dormant throughout the winter and summer germinate. These plants densely cover the whole area and start producing flowers towards the end of July The flowering is at its best in late August and early September After producing flowers most of these plants disappear by November Some of the annual herbs also appear in winters, but in summers the vegetation is very scanty due to low humidity high temperature and hot winds which have severe effect on vegetation

The habit of the plants varies The herbaceous plants constitute the major bulk of the flora, and these grow as weeds under natural conditions while most of the trees and shrubs are cultivated. Monocots are poorly represented forming only about 14% of the total number of species. Five dominant famil es of the area are Gramineae (36 species) Papilionaceae (26 species) Compositae (22 species) Euphorbiaceae (15 species) and Acanthaceae (13 species)

A list of the plants that have been found growing in Meerut College campus, is given below Beside one species of Gymnosperms 367 species of Angiosperms representing 284 genera from 91 families have been listed The sequence of families and genera is the same as adopted by Duthie in the Flora of the Upper Gangetic Plain. Hindi names of plants, where available, have also been given in brackets after botanical names Beside wild flora, cultivated trees shrubs and well established perennial plants have

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also been included in this list An attempt has been mad to bring th nomenclature up to date, only the valid name for each species being given.

DICOTYLEDONS

Ranunculaceae

Ranunculus sceleratus Linn
Clematis vitalba Linn.

Magnoliaceae

Michelia Champaca Linn. (Hindi Champa)

Anonaceae

Polyalthea longifolia Benth. & Hk. f (Hindi Asoki)
Anona squamosa Linn (Hindi Sharifa)
Artobotrys odoratissimus R Br

Menispermaceae

Tinospora cordifolia Miers (Hindi Gloe)
Cocculus hirsutus (Linn.) Diels (Hindi Hier)
Cissampelos pareira Linn. (Hindi Purhe)

Nymphaeaceae

Nymphaea lotus Linn (Hindi Kamal)

Papaveraceae

Argemone mexicana Linn (Hindi Peeli-kateli)
Fumaria indica Pugsley

Cruciferae

Nasturtium montanum Wall.
Coronopus didymus (Linn.) Sm.

Capparidaceae

Cleome viscosa Linn
Gynandropsis gynandra (Linn.) Briq (Hindi Hulhul)
Capparis sepiaria Linn (Hindi Taint)

Flacourtiaceae

Flacourtia indica (Burm f) Merr (Hindi Kango)

Caryophyllaceae

Saponaria vaccaria Linn.
Stellaria media Cyrill
Arenaria serpyllifolia Linn.
Spergula arvensis Linn
Polycarpaea corymbosa Lamk.

Portulacaceae

Portulaca oleracea Linn. (Hindi Kulfa)
Portulaca quadrifida Linn.

Malvaceae

Malva parviflora Linn.
Malvastrum tricuspidatum A. Gray
Sida veronicaefolia Lamk.
Sida rhombifolia Linn.
Sida cordifolia Linn.
Abutilon asiaticum G. Don. (Hindi Kanghi)
Malvaviscus arboreus Cav.
Hibiscus mutabilis Linn.
Hibiscus syriacus Linn.
Hibiscus rosa sinensis Linn. (Hindi Gudhal)
Hibiscus schizopetalus Hook. f.

Bombacaceae

Salmalia malabarica (D.C.) Schult. & Endl. (Hindi Semal)

Sterculiaceae

Melhania futteyporensis Munro ex Mast.
Melochia corchorifolia Linn.
Dombeya spectabilis Bojer

Tiliaceae

Triumfetta bartramia Linn.
Corchorus capsularis Linn.
Corchorus olitorius Linn.
Corchorus tridens Linn.
Corchorus aestuans Linn.

Malpighiaceae

Hiptage benghalensis (Linn.) Kurz (Hindi Madhavi lata)
Galphimia hirsuta Cav.

Zygophyllaceae

Tribulus terrestris Linn.

Oxalidaceae

Oxalis corniculata Linn. (Hindi Khatibuti)
Oxalis latifolia H. B. & K.
Oxalis martiana Zucc.

Rutaceae

Zanthoxylum alatum Roxb. (Hindi Tejbal)
Murraya paniculata (Linn.) Jack. (Hindi Kamini)
Murraya koenigii Spreng. (Hindi Mitha-neem)

Citrus medica Linn. (Hindi Nimbu)
Aegle marmelos Correa (Hindi Bel)

Meliaceae

Azadirachta indica Juss. (Hindi Neem)
Melia azedarach Linn (Hindi Bakain)

Rhamnaceae

Zizyphus mauritiana Lamk (Hindi Ber)

Vitaceae

Vitis vinifera Linn (Hindi Angur)

Sapindaceae

Dodonaea viscosa Jacq (Hindi Vilayati mehndi)

Anacardiaceae

Mangifera indica Linn (Hindi Am)

Moringaceae

Moringa oleifera Lamk. (Hindi Sanjna)

Papilionaceae

Crotalaria medicaginea Lamk.
Melilotus indica All.
Melilotus alba Desr
Trigonella polycerata Linn (Hindi Chini)
Trigonella corniculata Linn
Medicago lupulina Linn
Medicago denticulata Willd
Trifolium resupinatum Linn
Rhynchosia minima DC
Teramnus labialis Spreng
Psoralea corylifolia Linn
Sesbania sesban (Linn) Merr (Hindi Jait)
Sesbania bispinosa (Jacq) W F Wight (Hindi Jayant)
Tephrosia purpurea Pers
Indigofera linifolia Retz
Indigofera enneaphylla Linn
Indigofera tinctoria Linn (Hindi Neel)
Vicia sativa Linn
Lathyrus aphaca Linn
Abrus precatorius Linn (Hind Ratti Chaunti)
Dalbergia sissoo Roxb (Hindi Shisham)
Aeschynomene indica Linn. (Hindi Sola)
Alysicarpus vaginalis DC.

Desmodium triflorum DC.
Desmodium gangeticum DC.
Wisteria floribunda DC.

Caesalpinaceae

Cassia fistula Linn (Hindi Amaltas)
Cassia glauca Lamk.
Cassia nodosa Buch-Ham.
Cassia occidentalis Linn. (Hindi Kasaundi)
Cassia obtusifolia Linn
Bauhinia purpurea Linn.
Bauhinia variegata Linn (Hindi Kachnar)
Bauhinia acuminata Linn.
Tamarindus indica Linn. (Hindi Imli)
Caesalpinia pulcherrima Swartz.
Delonix regia (Boj.) Raf (Hindi Gulmohar)

Mimosaceae

Dichrostachys cinerea W & A.
Leucaena glauca Benth
Acacia nilotica (L.) Del (Hindi Babul)
Acacia catechu Willd. (Hindi Kher)
Acacia auriculiformis A. Cunn.
Albizzia odoratissima Benth (Hindi Siris)
Pithecolobium dulce Benth. (Hindi Jungle-jalebi)

Rosaceae

Prunus persica Stokes (Hindi Aru)
Prunus communis Huds. var. *insititia* (Hindi Alucha)
Rosa spp (Hindi Gulab)

Combretaceae

Terminalia arjuna Bedd. (Hindi Arjun)
Quisqualis indica Linn

Myrtaceae

Syzygium cumini (Linn.) Skeels (Hindi Jamun)
Syzygium cumini (Linn.) Skeels var. *microcarpa* Thwaites (Hindi Jamoa)
Psidium guajava Linn. (Hindi Amrud)
Eucalyptus citriodora Hook.
Callistemon lanceolatus Sweet.
Myrtus communis Linn.

Lythraceae

Lawsonia inermis Linn (Hindi Mehndi)
Lagerstroemia indica Linn.
Lagerstroemia speciosa (Linn.) Pers.

Punicaceae

Punica granatum Linn. (Hindi Anar)

Onagraceae

Jussiaea repens Linn

Caricaceae

Carica papaya Linn. (Hindi Papita)

Cucurbitaceae

Trichosanthes cucumerina Linn (Hindi Jangli chachinda)
Coccinia cordifolia (Linn.) cogn. (Hindi Kanduri)
Melothria maderaspatana Cogn (Hindi Gwal kakri)

Passifloraceae

Passiflora sp

Ficoideae

Trianthema portulacastrum Linn.
Trianthema decandra Linn.
Gisekia pharnaceoides Linn.

Cactaceae

Opuntia dillenii Haw (Hindi Nagphani)
Pereskia aculeata Mill
Nopalea cochinellifera Salm-Dyck.
Cereus schottii Engelm.

Umbelliferae

Centella asiatica (Linn.) Urban (Hindi Brahmi buti)

Araliaceae

Aralia sp.

Caprifoliaceae

Lonicera japonica Thunb
Sambucus canadensis Linn

Rubiaceae

Oldenlandia corymbosa Linn (*Hindi Daman-papar*)
Gardenia latifolia Ait
Coffea benghalensis Roxb
Mussaenda luteola Delila
Ixora coccinea Vahl.
Paederia foetida Linn

Compositae

Vernonia cinerea Less.
Ageratum conyzoides Linn.
Erigeron canadensis Linn
Blumea lacera DC.
Gnaphalium indicum Linn
Vicoa indica (Willd) DC
Vicoa vestita Benth
Pulicaria angustifolia DC.
Xanthium strumarium Linn. (Hindi Chota-datura)
Eclipta prostrata (Linn) Linn
Bidens biternata (Lour) Merr & Sherff
Tridax procumbens Linn
Artemisia scoparia Waldst & Kit.
Emilia sonchifolia DC.
Echinops echinatus Roxb
Cnicus arvensis Hoffm.
Volutarella ramosa (Roxb.) Santapau
Sonchus oleraceus Linn.
Sonchus arvensis Linn.
Launaea nudicaulis Hook. f
Carthamus oxyacantha Bieb
Eupatorium sp

Primulaceae

Anagallis arvensis Linn.

Myrsinaceae

Ardisia crenata Sims

Ebenaceae

Diospyros cordifolia Roxb. (Hindi Bistendu)

Oleaceae

Jasminum sambac Ait (Hind Motia Mogra)
Jasminum officinale Linn (Hindi Chameli.)
Jasminum undulatum Ker
Jasminum multiflorum (Burm. f.) Andr
Nyctanthes arbor-tristis Linn. (Hindi Harsingar)

Apocynaceae

Carissa carandas Linn. (Hindi Karaunda)
Alstonia scholaris R. Br (Hindi Satian)
Ervatamia coronaria Stapf (Hindi Chandni)
Wrightia sp
Nerium indicum Mill. (Hindi Kaner)

Thevetia peruviana (Pers.) K. Schum (Hindi Peeli Kaner)
Plumeria rubra Linn. forma *acutifolia* (Poir) Woodson (Hindi Gul-achin)

Beaumontia grandiflora Wall.

Asclepiadaceae

Calotropis procera R. Br (Hindi Ak, Madar)
Pergularia daemia (Forsk.) Chiov
Leptadenia reticulata W & A.
Cryptostegia grandiflora R. Br
Asclepias curassavica Linn.

Loganiaceae

Buddleia asiatica Lour (Hindi Dudhia)

Gentianaceae

Erythraea ramosissima Pers.

Boraginaceae

Cordia dichotoma Forst. f (Hindi Lasora)
Heliotropium eichwaldi Steud. ex. DC.
Heliotropium strigosum Willd

Convolvulaceae

Cuscuta reflexa Roxb (Hindi Amarbel)
Evolvulus alsinoides Linn.
Evolvulus nummularius Linn
Convolvulus pluricaulis Chois.
Convolvulus arvensis Linn.
Merremia dissecta Hallier f.
Ipomoea pestigridis Linn.
Ipomoea carnea Jacq (Hindi Hurhuck)
Ipomoea cairica (Linn.) Sweet. (Hindi Isk-pencha)
Argyreia speciosa Sweet.

Solanaceae

Solanum nigrum Linn. (Hindi Makoi)
Solanum xanthocarpum Schrad & Wendl (Hindi Kateli)
Solanum torvum Sw
Physalis minima Linn (Hindi Jangli-rasbherry)
Withania somnifera Dunal (Hindi Asgandh)
Datura innoxia Mill. (Hind Dhatura)
Nicotiana plumbaginifolia Viv
Cestrum nocturnum Linn. (Hind Rat ki-rani)
Cestrum diurnum Linn.

Scrophulariaceae

Antirrhinum orontium Linn
Mazus japonicus (Thunb.) O Kuntze
Lindernia crustacea (Linn.) F V Mueller
Lindernia ciliata (Colsm) Pennel
Veronica anagallis Linn
Veronica agrestis Linn.

Bignoniaceae

Bignonia venusta Ker
Bignonia unguis-cati Linn
Campsis grandiflora Loisel.
Tecoma stans DC.
Jacranda cuspidifolia Mart.

Acanthaceae

Thunbergia grandiflora Roxb
Ruellia sp
Dipteracanthus prostratus (Poir) Nees.
Hemigraphis hirta T Anders.
Daedalacanthus nervosus T Anders
Barleria prionitis Linn. (Hindi Kala barsa)
Justicia quinqueangularis Koen. ex Roxb
Justicia diffusa Willd.
Justicia simplex D Don
Justicia gendarussa Burm. (Hindi Jagat-madan)
Justicia secunda Vahl.
Peristrophe bicalyculata Nees.
Rungia pectinata (Linn.) Nees.

Verbenaceae

Lantana indica Roxb (Hindi Ghanari)
Phyla nodiflora (Linn.) Greene.
Clerodendrum indicum (Linn) Ktz.
Clerodendron speciosum D Ombrain
Stachytarpheta indica Vahl.
Duranta repens Linn
Petrea volubilis Linn.

Labiatae

Ocimum sanctum Linn. (Hindi Tulsi)
Leucas aspera Spreng (Hindi Ghumma)
Leucas cephalotes Spreng
Nepeta hindostana (Roth.) Haines
Salvia plebia R. Br

Nyctaginaceae

Boerhaavia diffusa Linn.
Boerhaavia repanda Willd.
Bougainvillea glabra Chois.
Bougainvillea spectabilis Willd.

Amarantaceae

Digera muricata (Linn.) Mart.
Amaranthus spinosus Linn.
Amaranthus viridis Linn.
Amaranthus polygamus Linn.
Aerva sanguinolenta (Linn.) Blume
Aerva lanata Juss.
Aerva javanica (Burm. f.) Spreng.
Achyranthes aspera Linn. (Hindi Chirchita)
Pupalia lappacea Juss.
Alternanthera sessilis R. Br.
Alternanthera repens (Linn.) O. Kuntze
Gomphrena celosioides Mart.

Chenopodiaceae

Chenopodium murale Linn.
Chenopodium ambrosioides Linn. (Hindi Khad-bathua)

Polygonaceae

Polygonum plebejum R. Br.
Polygonum glabrum Willd.
Rumex dentatus Linn.
Rumex hastatus Don
Antigonon leptopus Hook.

Aristalochiaceae

Aristolochia indica Linn.

Lauraceae

Cinnamomum sp.

Proteaceae

Grevillea robusta Cunn.

Loranthaceae

Dendrophthoe falcata (Linn. f.) Etting. (Hindi Banda)

Santalaceae

Santalum album Linn. (Hindi Chandan)

Euphorbiaceae

Euphorbia neriifolia Linn. (Hindi Thor Sehnur)
Euphorbia hypericifolia Linn.
Euphorbia hirta Linn.
Euphorbia thymifolia Linn.
Euphorbia geniculata Ortg
Euphorbia pulcherrima Willd.
Euphorbia tirucallii Linn.
Phyllanthus niruri Linn.
Croton bonplandianum Baill.
Acalypha wilkesiana Muell.
Ricinus communis Linn (Hindi Arand)
Jatropha gossypifolia Linn.
Manihot utillisima Pohl.
Excoecaria bicolor Hassk.
Codiaeum spp.

Cannabinaceae

Cannabis sativa Linn. (Hindi Bhang)

Moraceae

Morus laevigata Wall (Hindi Shatut)
Morus alba Linn (Hindi Tut)
Streblus asper Lour (Hindi Kuchna)
Ficus bengalensis Linn. (Hindi Bargad)
Ficus religiosa Linn (Hindi Peepal)
Ficus palmata Forsk. (Hindi Anjir)
• *Ficus glomerata* Roxb (Hindi Gular)
Ficus elastica Roxb
Ficus krishnae DC.
Broussonetia papyrifera Vent.

Urticaceae

Pouzolzia pentandra Benn.

Casuarinaceae

Casuarina equisetifolia Forst.

Ceratophyllaceae

Ceratophyllum demersum Linn.

GYMNOSPERMS

Cupressaceae

Thuja orientalis Linn (Hindi Morpankhi)

MONOCOTYLEDONS

Hydrocharitaceae

Hydrilla verticillata Royle.
Vallisneria spiralis Linn.

Cannaceae

Canna indica Linn. (Hindi Keli)

Musaceae

Musa paradisiaca Linn (Hindi Kela)
Ravenala madagascariensis Sonn.

Amaryllidaceae

Crinum latifolium Linn

Liliaceae

Asphodelus tenuifolius Cav (Hindi Jangli piazi)

Pontederiaceae

Eichhornia crassipes Solms. (Hindi Samunder sokh)

Araceae

Monstera deliciosa Liebm.
Colocasia antiquorum Schott. var *esculenta* Schott.

Commelinaceae

Commelina benghalensis Linn.
Murdannia nudiflorum (Linn) Brenan.
Cyano is axillaris Schult. f

Palmae

Roystonea regia O F Cook (Bottle palm)
Caryota urens Linn (Fish tail palm)
Livistona chinensis Br

Pandanaceae

Pandanus fascicularis Lamk. (Hindi Keora)

Lemnaceae

Spirodella polyrrhiza Schleld.
Lemna paucico tata Hegelm

Potamogetonaceae

Potamog ton crispus Linn.

Agavaceae

Agave sp

Cyperaceae

Cyperus diff us Linn
Cypere us Linn.
Cyperus ctundu Linn (Hindi Motha)
Cyp rus sculenta Linn. (Hindi Kaseru)
Cyper t iate Roxb
Fimbristyl s diphylla Vahl
Bulbo yl bar ata Kunth.

Gramineae

Dendrocalamus strictus Nees (Hindi Bans)
Poa annua Linn
Phragmites karka (Retz) Trin ex. Steud (Hindi Bansi)
Eragrostis poaeoides Beauv
Eragrostis pilosa (Linn.) Beauv
Eragrostis nigra Nees.
Eleusine indica (Linn.) Gaertn
Dactyloctenium aegyptium (Linn.) P Beauv (Hindi Makra)
Leptochloa filiformis Roem. & Sch
Sporobolus diander Beauv
Cynodon dactylon (Linn.) Pers (Hindi Dub)
Polypogon monspeliensis Desf.
Phalaris minor Retz
Perotis indica (Linn) O Ktze.
Tragus biflorus (Roxb) Schult.
Digitaria sanguinalis (Linn.) Scop
Panicum trypheron Schult. (Hindi Mujhri)
Echinochloa colonum (Linn.) Link (Hindi Sawan)
Paspalum scrobiculatum Linn.
Paspalidium flavidum (Retz.) Camus.
Urochloa panicoides Beauv
Oplismenus burmanii (Retz) P Beauv
Brachiaria reptans (Linn.) Gard & Hubb
Brachiaria ramosa (Linn.) Stapf.
Setaria palmifolia (Koenig) Stapf
Setaria glauca (Linn.) P Beauv
Setaria tomentosa (Roxb.) Kunth
Cenchrus ciliaris Linn. (Hindi Anjana)
Cenchrus catharticus Del.
Imperata cylindrica (Linn.) P Beauv
Saccharum spontaneum Linn. (Hindi Kans)
Erianthus munja (Roxb) Jeswelt (Hindi Munj)
Apluda mutica Linn. (Hindi Bhanjuri)
Sorghum halepense (Linn.) Pers. (Hindi Banchari)
Dichanthium annulatum (Forsk.) Stapf. (Hindi Bare jargi)
Bothriochloa pertusa (Willd.) A. Camus

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EFFECT OF ANIONS ON THE SORPTION OF POTASH IN SOIL

A. N Pathak and K. N Sharma
Agricultural Chemistry Section, Govt. Agricultural College Kanpur

The term Sorption is employed in a restricted sense It emplies merely that the process is one of absorption, adsorption or both In a broader sense this process is deeply related to the cation exchange phenomenon responsible for K-fixation in different soils

Ayres (1941) observed that the sorption of potash and ammonium incre ases with the increase in degree of Ca-saturation and decreases with the decrease in concentration of the cations In addition to this the sorption of K and NH_4 was much higher from SO_4^{--} than from the Cl^- of these cations. Wiklander (1950) inferred that K^- fixation was very low when clay was saturated with H^+ K^+ or NH_4^+ but markedly high when saturated with Ca^{++} and especially with Na.

Wiegner (1931) pointed out that on the addition of symmetry quantities of KCl and CH_3COOK to H-saturated bentonite, the fixation of K from CH_3COOK was greater than from KCL Joffe and Levine (1947) observed that when potassium acetate was added 17 7 mg K was fixed as compared to 13 7 mg K with KCl Similar differences in the amount of K fixed with different potash salts have been recorded by De Turk Wood and Bray (1943) They observed that the addition of K as phosphate results in greater fixation than as chloride Joffe and Kolodny (1936) attributed greater fixation of K in the B horizon to a large number of phosphate complexes which act as agents of K-fixation. Sturgis and Moore (1939) also observed different K-fixation with KCl and K_2HPO_4

Ayres and Hagihara (1953) observed that the ability of soils to retain K from KCl was very slight from K_2SO_4 considerable and from various forms of potassic phosphates marked They observed that the losses of K from KCl by leaching were smaller when KCl was applied to the soil with $(NH_4)_2HPO_4$ than when used alone. Mc. Inture, Shaw and Robinson (1955) summarized that recoveries of K from K_2PO_4 and K_2SO_4 were fairly uniform for 200 lbs. annual inputs of K_2O but recoveries from 1000 lbs inputs as K_2PO_4 were almost twice than those from the corresponding incorporation of K_2SO_4 Mehta and Shah (1956) reported that K fixation with KCl and K_2SO_4 was about equal and was less than with KH_2PO Karim and Malek (1957) made studies on East Pakistan soils under different conditions and concluded that clay loam, loam, silt loam and loamy sand fixed on an average 56.5%, 61.8% 69 1% and 35% respectively of added K as K_2SO KCl KNO_3 and K_2CO after 60 days. An excellent review of the subject has recently been given by Agarwal (1960)

TABLE 1

Potash-sorption as affected by different anions

Soil Depth	0"–6"			6"–12"			12"– [illegible]		
Treatments	K added/100 gms soil	K recovered/100 gms soil	K fixed over control	K added/100 gms soil	K recovered/100 gms soil	K fixed over control	K added/100 gms soil	K recovered/100 gms soil	K [illegible]
	gm	gm	%	gm	gm		gm	gm	%
				(a) *Normal cultivated Soil*					
K Cl	1 504	1 168	42 82	1 276	1 213	14 15	1 901	[illegible]	[illegible]
K_2SO_4	1 504	0 960	56 65	1 276	1 019	29 39	1 901	1 [illegible]	[illegible]
K_3PO_4	1 504	0 708	73 42	1 276	0 560	65 34	1 901	[illegible]	[illegible]
$K_2H PO_4$	1 504	0 736	1 35	1 276	0 707	53 81	1 901	[illegible]	[illegible]
$KH_2 PO_4$	1 504	1 198	40 83	1 276	0 781	48 05	1 901	0 [illegible]	[illegible]
Control	Nil	0 308	Nil	Nil	0 118	Nil	Nil	[illegible]	[illegible]
				(b) *Hydrogen saturated Soil*					
KCl	1 504	0 871	50 80	1 276	1 108	25 79	1·901	1 512	[illegible]
K_2SO_4	1 504	0 722	60 71	1 276	0 909	49 21	1·901	[illegible]	[illegible]
K_3PO_4	1 504	0 589	69 55	1 276	0 502	77 93	1 901	0 [illegible]	[illegible]
K_2HPO_4	1 504	1 068	37 70	1 276	0·826	52 59	1 901	0 [illegible]	[illegible]
KH_2PO_4	1 504	1 123	31 05	1 276	1 112	30 19	1 901	0 [illegible]	[illegible]
Control	Nil	0 131	Nil	Nil	0 221	Nil	Nil	[illegible]	[illegible]
				(c) *Calcium saturated Soil*					
KCl	1 [illegible]	1 105	38 17	1 276	0 [illegible]	36 29	1·901	[illegible]	[illegible]
K_2SO_4	1 [illegible]	0 840	55 79	1 276	0 [illegible]	46 [illegible]	[illegible]	[illegible]	[illegible]
K_3PO_4	1 501	0 [illegible]	83 32	1 2[illegible]6	0 537	[illegible] 37	[illegible]	[illegible]	[illegible]
K_2HPO_4	1 504	0 601	71 45	1 276	0 [illegible]	61 [illegible]	[illegible]	[illegible]	[illegible]
KH_2PO_4	1 504	0 896	5[illegible] 73	1 276	0 8[illegible]	35 63	[illegible]	[illegible]	[illegible]
Control	Nil	0 17	Nil	Nil	0 0[illegible]	Nil	Nil	[illegible]	[illegible]

Material and Method

Soil for the study was a normal cultivated soil taken from plot no. 193 students Instructional Farm Govt Agricultural College Kanpur (U P) Hydrogen and calcium saturated soils were prepared by leaching For studying K-Sorption different potassic salts like KCl K_2SO_4 K_3PO_4 KH_2PO_4 and K_2HPO_4 were added equal to 1.5 times cation exchange capacity of the soil and dried continuously at 105°C for 6 hours Potash retention was determined by leaching the soil with N $BaCl_2$ Similarly Potash retention in combination with Ammophos and Potassium chloride and with superphosphate and potassium chloride in the ratio of 1 1 1:2 and 2.1 was determined

Chemical analysis was done by the standard methods as given by Piper (1950)

Table 2

Phosphorus retention as affected by different potassic phosphate

Soil Depth	0"—6"			6"—12"			1"—24		
Treatments	P added/100 gms soil	P recovered/100 gm soil	P fixed of added over control	P added/100 gms soil	P recovered/100 gm soil	P fixed of added over control	P added/100 gms soil	P recovered/100 gms soil	P fixed of added over control
	gm	gm	%	gm.	gm	%	gm	gm	%
				( ) *Normal cultivated Soil*					
K_3PO_4	0 398	0 008	97 97	0 338	0 0269	9* 05	0 525	0·00809	98 46
K_2HPO_4	0·587	0 0108	96 20	0 506	0 03507	93 05	0 788	0 01733	97 78
KH_2PO_4	1 194	0 *617	78 09	1 015	0 4776	52 86	1 576	0 63949	59 43
				(b) *Hydrogen saturated Soil*					
K_3PO_4	0 398	Nil	100	0 338	0 010*9	96 81	0 525	Nil	100
K_2HPO_4	0 597	0 0512	91 42	0 506	0·1592	68 55	0 788	0 0557	93 73
KH_2PO_4	1 194	0 2483	79 21	1·015	0 61251	39 54	1 576	0 74473	5.. 75
				( ) *Calcium saturated Soil*					
K_3PO_4	0 398	Nil	100	0 338	0 0081	97 58	0 525	Nil	100
K_2HPO_4	0 597	Nil	100	0 506	0 0162	96 80	0 788	0 004047	99·48
KH_2PO_4	1 194	0 1525	85 92	1 015	0 45601	54 99	1 576	0 57878	63 28

TABLE 3

Potash-sorption from KCl in combination with $(NH_4)_2$ HP O_4 and [illegible]

Soil Depth		0"–6"			6"–12"			12"–[illegible]		
Fertiliser Mixtures (Ratio)		K added/100 gms soil	K recovered/100 gms soil	K fixed of added over control	K added/100 gms soil	K recovered/100 gms soil	K fixed of added over control	K added/100 gms soil	K recovered/100 gms soil	K fixed of added over control
		gm	gm	%	gm	gm	%	gm	gm	
				( ) *Colloidal Soil*						
$(NH_4)_2$ HPO_4+KCl	1:1	1 504	1 049	50 76	1 276	1 1[illegible]	1 16	[illegible]	1 25[illegible]	[illegible]
$(NH_4)_2$ HPO_4+KCl	1:2	3 008	2 400	30 46	2 552	2 370	19 1[illegible]	3 9[illegible]	[illegible]	[illegible]
$(NH_4)_2$ HPO_4+KCl	2:1	1 504	0 841	65 90	1 276	1 000	30 5[illegible]	[illegible]	0 811	[illegible]
Superphosphate+KCl	1:1	1 504	1 317	30 92	1 276	1 257	10 7[illegible]	[illegible]	[illegible]	[illegible]
Superphosphate+KCl	1:2	3 008	2 701	20 35	2 552	2 4[illegible]	9 80	3 9[illegible]	2 2[illegible]	[illegible]
Superphosphate+KCl	2:1	1 504	1 332	31 92	1 276	1 215	14 11	[illegible]	[illegible]	[illegible]
Control		Nil	0 308	Nil	Nil	0 118	Nil	Nil	0 1[illegible]	[illegible]
				(I) *Hydrogen saturated Soil*						
$(NH_4)_2$ HPO_4+KCl	1:1	1 504	1 37[illegible]	17 16	1 276	1 064	33 94	[illegible]	[illegible]	[illegible]
$(NH_4)_2$ HPO_4+KCl	1:2	3 008	1 680	41 6[illegible]	2 55[illegible]	2 437	32 4[illegible]	3 [illegible]	2 [illegible]	[illegible]
$(NH_4)_2$ HPO_4+KCl	2 1	1 504	0 030	46 88	1 2[illegible]6	0 930	44 44	[illegible]	[illegible]	[illegible]
Superphosphate+KCl	1:1	1 504	1 320	1[illegible] 24	1 [illegible]76	1 [illegible]11	[illegible]	[illegible]	[illegible]	[illegible]
Superphosphate+KCl	1:2	3 008	2 871	8 10	2 55[illegible]	2 337	9 25	3 [illegible]	2 [illegible]	[illegible]
Superphosphate+KCl	2 1	1 504	1 10[illegible]	31 06	1 [illegible]76	1 10[illegible]	5 [illegible]	[illegible]	[illegible]	[illegible]
Control		Nil	0 131	Nil	Nil	0 221	Nil	Nil	[illegible]	
				( ) *Cation saturated Soil*						
$(NH_4)_2$ HPO_4 KCl	1 1	1 504	1 091	58 90	1 [illegible]	0 [illegible]	[illegible]	[illegible]	[illegible]	[illegible]
$(NH_4)_2$ HPO_4+KCl	1 2	3 008	2 5[illegible]	46 53	2 55[illegible]	1 [illegible]	41 04	3 [illegible]	[illegible]	[illegible]
$(NH_4)_2$ HPO_4+KCl	2 1	1 [illegible]04	0 511	77 4[illegible]	1 [illegible]	0 4[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Superphosphate+KCl	1:1	1 504	1 392	21 0[illegible]	1 [illegible]	0 7[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Superphosphate KCl	1[illegible]	3 008	2 610	12 51	[illegible]	2 5[illegible]	11 [illegible]	3 9[illegible]	[illegible]	[illegible]
Superphosphat +KCl	2 1	1 504	1 30[illegible]	25 0[illegible]	1 2[illegible]	1 04	[illegible]	[illegible]	[illegible]	[illegible]
Control		Nil	0 1[illegible]	Nil	Nil	0 073	Nil	Nil	[illegible]	

Experimental Result and Discussion

It is observed from the data in table 1 that potash as phosphates showed highest retention in the soil followed by as sulphate and as chloride which may be attributed to the sorption of SO_4^{--} by soil colloidal material more strongly held than Cl^- but less strongly than PO_4^{---} Stout (1940) and Dickman and Bray (1941) suggested the theory of phosphate-linkage which act as agents of K-fixation in the soil. Mattson and Wiklander (1940) found potash to fix more strongly as SO_4^{--} than as Cl^- Calcium saturated soil showed greater sorption of K as phosphate than the normal and hydrogen soils This may be attributed to lesser adsorption energy of Ca than H as also observed by Ayres (1941)

It is further observed that K_3PO_4 fixed more phosphorus than K_2HPO_4 or KH_2PO_4 (Table 2) the values were still higher in H and Ca saturated soils than in the normal. This may be attributed to the precipitation of iron and aluminium-phosphates in hydrogen soil and calcium phosphate in the calcium soil. Ayres and Hagihara also observed a similar phenomenon

Data in table 3 indicate that phosphates are instrumental in the retention of K from KCl added to the soil which was highest with $(NH_4)_2HPO_4$ and KCl 2:1 ratio Calcium soils are more effective in K fixation than hydrogen or normal soils. It can be inferred that superphosphate and KCl if applied in 1 1 or 2 1 ratio, the satisfactory release of K from the soil colloid is vigorous and shows no effect of superphosphate on the retention of K in the soil.

Summary

Effect of different anions on potash fixing power of the soil was studied in normal cultivated, hydrogen saturated and calcium saturated soils It was observed that potash as phosphates showed greater fixation than as SO_4^{--} or Cl^- Among the different phosphates K_3PO_4 showed highest fixation simultaneously there was fixation of PO_4 as well

Calcium saturated soil showed greater fixation as compared to H saturated or normal soil. There was greater retention of K when applied as KCl in combination with ammophos than when applied as KCl alone

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ON THE EXPANSIONS OF HYPERGEOMETRIC FUNCTIONS

R. C. PANDEY

D. A. V. College, Kanpur

1. J. L. Burchnall and T. W. Chaundy [2] in the year 1940 gave the various expansions of Appell's hypergeometric functions F_1, F_2, F_3 and F_4. They made use of the inverse pair of symbolic operators

$$\nabla_{x,y}(h) = \frac{\Gamma(h)\,\Gamma(\delta+\delta'+h)}{\Gamma(\delta+h)\,\Gamma(\delta'+h)}$$

and

$$\Delta_{x,y}(h) = \frac{\Gamma(\delta+h)\,\Gamma(\delta'+h)}{\Gamma(h)\,\Gamma(\delta+\delta'+h)}$$

where δ and δ' stand for $x\frac{\partial}{\partial x}$ and $y\frac{\partial}{\partial y}$ respectively. They also extended the results to functions of higher order (*i.e.* with more parameters) in two variables. In this paper I have tried to find the expansions of their type in hypergeometric functions of three variables recently defined by Saran [4] which are as follows :—

$$F_E(\alpha_1, \alpha_1, \alpha_1, \beta_1, \beta_2, \beta_2;\ \gamma_1, \gamma_2, \gamma_3;\ x, y, z)$$

$$= \sum \frac{(\alpha_1, m+n+p)(\beta_1, m)(\beta_2, n+p)}{(1, m)(1, n)(1, p)(\gamma_1, m)(\gamma_2, n)(\gamma_3, p)}\, x^m y^n z^p$$

$$r+(\sqrt{s}+\sqrt{t})^2=1$$

$$F_F(\alpha_1, \alpha_1, \alpha_1, \beta_1, \beta_2, \beta_1;\ \gamma_1, \gamma_2, \gamma_2;\ x, y, z)$$

$$= \sum \frac{(\alpha_1, m+n+p)(\beta_1, m+p)(\beta_2, n)}{(1, m)(1, n)(1, p)(\gamma_1, m)(\gamma_2, n+p)}\, x^m y^n z^p$$

$$rs=(1-s)(s-t)$$

$$F_G(\alpha_1, \alpha_1, \alpha_1, \beta_1, \beta_2, \beta_3;\ \gamma_1, \gamma_2, \gamma_2;\ x, y, z)$$

$$= \sum \frac{(\alpha_1, m+n+p)(\beta_1, m)(\beta_2, n)(\beta_3, p)}{(1, m)(1, n)(1, p)(\gamma_1, m)(\gamma_2, n+p)}\, x^m y^n z^p$$

$$r+s=1,\quad r+t=1$$

$$F_K(\alpha_1, \alpha_2, \alpha_2, \beta_1, \beta_2, \beta_1;\ \gamma_1, \gamma_2, \gamma_3;\ x, y, z)$$

$$= \sum \frac{(\alpha_1, m)(\alpha_2, n+p)(\beta_1, m+p)(\beta_2, n)}{(1, m)(1, n)(1, p)(\gamma_1, m)(\gamma_2, n)(\gamma_3, p)}\, x^m y^n z^p$$

$$t=(1-r)(1-s)$$

$$F_M(\alpha_1, \alpha_2, \alpha_2, \beta_1, \beta_2, \beta_1;\ \gamma_1, \gamma_2, \gamma_2;\ x, y, z)$$

$$= \sum \frac{(\alpha_1, m)(\alpha_2, n+p)(\beta_1, m+p)(\beta_2, n)}{(1, m)(1, n)(1, p)(\gamma_1, m)(\gamma_2, n+p)}\, x^m y^n z^p$$

$$r+t=1,\ s=1$$

$$F_N(\alpha_1, \alpha_2, \alpha_3, \beta_1, \beta_2, \beta_1;\ \gamma_1, \gamma_2, \gamma_2;\ x, y, z)$$

$$= \sum \frac{(\alpha_1, m)(\alpha_2, n)(\alpha_3, p)(\beta_1, m+p)(\beta_2, n)}{(1, m)(1, n)(1, p)(\gamma_1, m)(\gamma_2, n+p)}\, x^m y^n z^p,$$

$$s(1-r)+t(1-s)=0$$

$$F_P(\alpha_1, \alpha_2, \alpha_1, \beta_1, \beta_1, \beta_2;\ \gamma_1, \gamma_2, \gamma_2;\ x, y, z)$$

$$= \sum \frac{(\alpha_1, m+p)(\alpha_2, n)(\beta_1, m+n)(\beta_2, p)}{(1, m)(1, n)(1, p)(\gamma_1, m)(\gamma_2, n+p)}\, x^m y^n z^p$$

$$4\,rst=(st-s-t)^2$$

$$F_R(\alpha_1, \alpha_2, \alpha_1, \beta_1, \beta_2, \beta_1;\ \gamma_1, \gamma_2, \gamma_2;\ x, y, z)$$

$$= \sum \frac{(\alpha_1, m+p)(\alpha_2, n)(\beta_1, m+p)(\beta_2, n)}{(1, m)(1, n)(1, p)(\gamma_1, m)(\gamma_2, n+p)}\, x^m y^n z^p$$

$$s(1-\sqrt{r})^2+t(1-s)=0$$

$$F_S(\alpha_1, \alpha_2, \alpha_2, \beta_1, \beta_2, \beta_3;\ \gamma_1, \gamma_1, \gamma_1;\ x, y, z)$$

$$= \sum \frac{(\alpha_1, m)(\alpha_2, n+p)(\beta_1, m)(\beta_2, n)(\beta_3, p)}{(1, m)(1, n)(1, p)\ (\gamma_1, m+n+p)}\, x^m y^n z^p$$

$$1/r+1/s=1,\ s=t$$

and

$$F_T(\alpha_1, \alpha_2, \alpha_2, \beta_1, \beta_2, \beta_1;\ \gamma_1, \gamma_1, \gamma_1;\ x, y, z)$$

$$= \sum \frac{(\alpha_1, m)(\alpha_2, n+p)(\beta_1, m+p)(\beta_2, n)}{(1, m)(1, n)(1, p)\ (\gamma_1, m+n+p)}\, x^m y^n z^p$$

$$t=r-rs+s$$

where

$(\alpha, m)=\alpha(\alpha+1) \ldots (\alpha+m-1)$, $(\alpha, 0)=1$

and $|x|<r$, $|y|<s$ and $|z|<t$.

The summation in the above triple series extends over all positive integral values of m, n and p from zero to infinity.

Now using the above operators it is easy to get the following operational relations —

$$1 \begin{cases} F_K(\alpha_1, \alpha_2, \alpha_2, \beta_1, \beta_2, \beta_1;\ \gamma_1, \gamma_2, \gamma_3;\ x, y, z) \\ (1.1) = \nabla x\, z\, (\beta_1)\, F(\alpha_1, \beta_1;\ \gamma_1;\ x)\, F_2(\alpha_2, \beta_2, \beta_1;\ \gamma_2, \gamma_3;\ y, z) \\ F(\alpha_1, \beta_1;\ \gamma_1;\ x)\, F_2(\alpha_2, \beta_2, \beta_1;\ \gamma_2, \gamma_3;\ y, z) \\ (1.2) = \Delta x\, z\, (\beta_1) \times F_K(\alpha_1, \alpha_2, \alpha_2, \beta_1, \beta_2, \beta_1;\ \gamma_1, \gamma_2, \gamma_3;\ x, y, z) \end{cases}$$

$$= F_P(\alpha_1\ \alpha_1\ \alpha_2\ \beta_1\ \beta_2\ \beta_1\ \ \gamma_1\ \gamma_2\ \gamma_1\ \ x\ y\ \ )$$

II

$$(1\cdot3) = \nabla_{x\ y}(\alpha_1) \times$$

$$\times F^*_N([\ _1]\ [\alpha_1]\ \alpha_2\ \beta_1\ \beta_2\ \beta_1\ \ \gamma_1\ \gamma_2\ \gamma_2\ \ x\ y\ z)$$

$$F_N([\alpha_1]\ [\alpha_1]\ \alpha_2\ \beta_1\ \beta_2\ \beta_1\ \ \gamma_1\ \gamma_1\ \gamma_2\ \ x\ y\ z)$$

$$(1\cdot4) = \Delta_{x\ y}(\alpha_1) \times$$

$$\times F_P(\ _1\ \alpha_1\ \alpha_2\ \beta_1\ \beta_2\ \beta_1\ \ \gamma_1\ \gamma_2\ \gamma_2\ \ x\ y\ z)$$

III

$$F_E(\alpha_1, \alpha_1\ \alpha_1\ \beta_1\ \beta_2\ \beta_2\ \ \gamma_1\ [\gamma_2]\ [\gamma_2]\ \ x\ y\ z)$$

$$(1\cdot5) = \nabla_{y\ z}(\gamma_2)\ F_2(\alpha_1\ \beta_1\ \beta_2\ \ \gamma_1\ \gamma_2\ \ x\ y+z)$$

$$F_2(\alpha_1\ \beta_1\ \beta_2\ \ \gamma_1\ \gamma_2\ \ x\ y+z)$$

$$(1\cdot6) = \Delta_{y\ z}(\gamma_2) \times$$

$$\times F_E(\alpha_1, \alpha_1\ \alpha_1\ \beta_1\ \beta_2\ \beta_2\ \ \gamma_1\ [\gamma_2]\ [\gamma_2]\ \ x\ y\ z)$$

Similar other relations can also be written for Saran s functions including Lauricella s functions [3]

2. Now we know from Burchnall and Chaundy [2] that

(i) $$\frac{1}{(\delta+c\ r)}\ F(a, b\ \ c\ \ x) = \frac{1}{(c\ r)}\ F(a\ \ b\ \ c+r\ \ x)$$

(ii) $$(-\delta\ r)\ F(a, b\ \ c\ \ x)$$

$$= (-)^r\ \frac{(a\ r)(b\ r)}{(c\ r)}\ x^r\ \ F(a+r\ \ b+r\ \ c+r\ \ x)$$

and $\frac{1}{(-a-\delta-\delta'+1\ \ r)}$ changes the parameter $(a, m+n)$ in the Appell's functions, into

(iii) $$(-)^r\ \frac{(a, r)(a-r\ \ m+n)}{(a-r\ \ 2r)}$$

Also the operators $\nabla_{x\ y}(h)$ and $\Delta_{x\ y}(h)$ can be written in the form of series [2] viz,

$$(2.1)\quad \nabla_{x\ y}(h) = \sum_{r=0}^{\infty}\ \frac{(-\delta\ r)(-\delta'\ r)}{(1\ r)(h, r)}$$

$$(2.2)\quad \Delta_{x\ y}(h) = \sum_{r=0}^{\infty}\ \frac{(-\delta, r)(-\delta'\ r)}{(1\ r)(-h-\delta-\delta'+1\ \ r)}$$

$$(2.3)\quad = \sum_{r=0}^{\infty}\ (-)\ \ \frac{(h, 2r)(-\delta\ r)(-\delta'\ \ )}{(1\ r)(h+r-1\ \ r)(\delta+h, r)(\delta'+h, r)}$$

Type I

Now it is easy to get by using (2.1) in (1 1) that

$$F_K(\alpha_1, \alpha_2, \alpha_2, \beta_1\ \beta_2, \beta\ \ \ \gamma_1\ \gamma_2, \gamma\ \ \ x\ y\ z)$$

The parameters within square brackets indicate the presence of $(\ _1, m) \times (\alpha_1\ \ )$ in the right hand sum ([4] page 77)

$$=\sum_{r=0}^{\infty}\frac{(-\delta,r)(-\delta',r)}{(1,r)(\beta_1,r)}F(\alpha_1,\beta_1;\gamma_1;x)\times$$

$$\times F_2(\alpha_2,\beta_2,\beta_1;\gamma_2,\gamma_3;y,z)$$

$$=\sum_{m=0}^{\infty}\sum_{n,p=0}^{\infty}\sum_{r=0}^{\infty}\frac{(-m,r)(-n,r)}{(1,r)(\beta_1,r)}\frac{(\alpha_1,m)(\beta_1,m)}{(1,m)(\gamma_1,m)}x^m\frac{(\alpha_2,n+p)(\beta_2,n)(\beta_1,p)}{(1,n)(1,p)(\gamma_2,n)(\gamma_3,p)}y^n z^p$$

$$(2.4)=\sum_{r=0}^{\infty}\frac{(\alpha_1,r)(\alpha_2,r)(\beta_1,r)}{(1,r)(\gamma_1,r)(\gamma_2,r)}x^r y^r\times$$

$$\times F(\alpha_1+r,\beta_1+r;\gamma_1+r;x)\,F_2(\alpha_2+r,\beta_2,\beta_1+r;\gamma_2,\gamma_3+r;y,z)$$

and by using (2.2) in (1.2) that

$$F(\alpha_1,\beta_1;\gamma_1;x)\,F_2(\alpha_2,\beta_2,\beta_1;\gamma_2,\gamma_3;y,z)$$

$$=\sum_{r=0}^{\infty}\frac{(-\delta,r)(-\delta,r)}{(1,r)(-\beta_1-\delta-\delta+1,r)}\times$$

$$\times F_K(\alpha_1,\alpha_1,\alpha_2,\beta_1,\beta_2,\beta_1;\gamma_1,\gamma_2,\gamma_3;x,y,z)$$

$$=\sum_{m,n,p=0}^{\infty}\sum_{r=0}^{\infty}\frac{(-m,r)(-n,r)}{(1,r)(-\beta_1-m-n+1,r)}\times\frac{(\alpha_1,m)(\alpha_2,n+p)(\beta_1,m+p)(\beta_2,n)}{(1,m)(1,n)(1,p)(\gamma_1,m)(\gamma_2,n)(\gamma_3,p)}x^m y^n z^p$$

$$(2.5)=\sum_{r=0}^{\infty}(-)^r\frac{(\alpha_1,r)(\alpha_2,r)(\beta_1,r)}{(1,r)(\gamma_1,r)(\gamma_2,r)}x^r z^r\times$$

$$\times F_K(\alpha_1+r,\alpha_2+r,\alpha_2+r,\beta_1+r,\beta_2,\beta_1+r;\gamma_1+r,\gamma_2,\gamma_3+r;x,y$$

Type II

Again from (2.1) and (1.3) we get

$$F_P(\alpha_1,\alpha_1,\alpha_2,\beta_1,\beta_2,\beta_1;\gamma_1,\gamma_2,\gamma_3;x,y,z)$$

$$=\sum_{r=0}^{\infty}\frac{(-\delta,r)(-\delta,r)}{(1,r)(\alpha_1,r)}\times$$

$$\times F_N([\alpha_1],[\alpha_1],\alpha_2,\beta_1,\beta_2,\beta_1;\gamma_1,\gamma_2,\gamma_3;x,y,z)$$

$$(2.6)=\sum_{r=0}^{\infty}\frac{(\alpha_1,r)(\beta_1,r)(\beta_2,r)}{(1,r)(\gamma_1,r)(\gamma_2,r)}x^r y^r\times$$

$$\times F_N([\alpha_1+r],[\alpha_1,r],\alpha_2,\beta_1+r,\beta_2+r,\beta_1+r;\gamma_1+r,\gamma_2+r,\gamma_3+r;x,y,z$$

and from (2.2) and (1.4) that

$$F_N([\alpha_1],[\alpha_1],\alpha_2,\beta_1,\beta_2,\beta_1;\gamma_1,\gamma_2,\gamma_3;x,y,z)$$

$$=\sum_{r=0}^{\infty}\frac{(-\delta,r)(-\delta,r)}{(1,r)(-\alpha_1-\delta-\delta+1,r)}\times$$

$$\times F_P(\alpha_1,\alpha_1,\alpha_2,\beta_1,\beta_2,\beta_1;\gamma_1,\gamma_2,\gamma_3;x,y,z)$$

$$(2.7) = \sum_{r=0}^{\infty} (-)^r \frac{(\ _1\ r)(\beta_1\ r)(\beta_3, r)}{(1\ r)(\gamma_1\ r)(\gamma_3, r)} x^r y^r \times$$
$$\times F_P(a_1+r\ a_1+r\ a_3, \beta_1+r\ \beta_2+r\ \beta_1+r$$
$$\gamma_1+r\ \gamma_2+r\ \gamma_3+r\ x\ y\ z)$$

Type III

Further by using (2.1) in (1.5) we can get

$$F_E(a_1\ a_1\ a_1\ \beta_1\ \beta_2, \beta_3\ \gamma_1, [\gamma_2]\ [\gamma_2]\ x\ y\ z)$$
$$= \sum_{r=0}^{\infty} \frac{(-\delta, r)(-\delta'\ r)}{(1\ r)(\gamma_2, r)} \times$$
$$\times F_2(a_1\ \beta_1\ \beta_2\ \gamma_1, \gamma_2\ x\ y+z)$$
$$(2.8) = \sum_{r=0}^{\infty} \frac{(a_1\ 2\ )(\beta_2, 2r)}{(1\ r)(\gamma_2, r)(\gamma_2, 2r)} y^r z^r \times$$
$$\times F_2(\ _1+2r\ \beta_1\ \beta_2+2r\ \gamma_1\ \gamma_2+2r\ x\ y+z)$$

and using (2.3) in (1.6) that

$$F_2(a_1\ \beta_1\ \beta_2\ \gamma_1\ \gamma_2\ x\ y+z)$$
$$= \sum_{r=0}^{\infty} (-)^r \frac{(\gamma_2, 2r)(-\delta\ r)(-\delta\ r)}{(1\ \ )(\gamma_2+r-1\ r)(\delta+\gamma_2, r)(\delta+\gamma_2, r)} \times$$
$$\times F_E(a_1, a_1\ \ _1\ \beta_1\ \beta_2, \beta_2\ \gamma_1\ [\gamma_2]\ [\gamma_2]\ x\ y\ z)$$
$$= \sum_{r=0}^{\infty} (-)^r \frac{(a_1\ 2r)(\beta_2\ 2r)}{(1\ r)(\gamma_2+r-1\ r)(\gamma_2, 2r)} y^r z^r \times$$
$$\times F_E(a_1+2r\ a_1+2r\ a_1+2r\ \beta_1\ \beta_2+2r\ \beta_2+2r$$
$$\gamma_1\ [\gamma_2+2r]\ [\gamma_2+2r]\ x\ y\ z)$$

3 In this article an attempt has been made to find out the expansions of Saran's [4] and Lauricella's [3] hypergeometric functions of three variables in double series by using the inverse pair of symbolic operators namely

$$\nabla_{x\ yz}(h) = \frac{\Gamma(h)\ \Gamma(\delta_1+\delta_2+\delta_3+h)}{\Gamma(\delta_1+h)\ \Gamma(\delta_2+\delta\ +h)}$$

and

$$\Delta_{x\ yz}(h) = \frac{\Gamma(\delta_1+h)\ \Gamma(\delta\ +\delta\ +h)}{\Gamma(h)\ \Gamma(\delta_1+\delta_2+\delta_3+h)}$$

where δ_1, δ_2 and δ_3 stand for $x\frac{\partial}{\partial x}$, $y\frac{\partial}{\partial y}$ and $z\frac{\partial}{\partial z}$ respectively

Using the above operators we can write as in article 2 the following two types of relations —

I
$$F_A(a_1\ a_1\ a_1\ \beta_1\ \beta_2, \beta\ \ \gamma_1, \gamma_2, \gamma_3\ x\ y\ z)$$
$$(3.1) = \nabla_{x, yz}(a_1)\ F(\ _1\ \beta_1\ \gamma\ \ x)\ F_2(a_1\ \beta_2, \beta_3\ \gamma_2, \gamma_3\ y\ z)$$
$$F(a_1\ \beta_1\ \gamma_1\ x)\ F_2(a_1\ \beta_2, \beta_3\ \gamma_2, \gamma\ \ y\ z)$$
$$(3.2) = \Delta_{x\ yz}(a_1) \times$$
$$\times F_A(a_1\ a_1, a_1\ \beta_1\ \beta_2, \beta_3\ \gamma_1, \gamma_2, \gamma_3\ x, y\ z)$$

$$= F_2(\beta_1\ a_1\ a_2\ \gamma_1\ \gamma_2\ x\ y+z)$$

$$(3.3)\quad = \nabla_{y\ xz}(\beta_1)\times$$

II $\quad \times F_M(a_1\ a_2, a_2\ \beta_1\ [\beta_1]\ \beta_1\ \gamma_1\ \gamma_2, \gamma_2\ x\ y\ )$

$$F_M(a_1\ a_2\ a_2, \beta_1\ [\beta_1]\ \beta_1\ \gamma_1\ \gamma_2, \gamma_2\ x\ y\ z)$$

$$(3.4)\quad = \Delta_{y\ xz}(\beta_1)\ F_2(\beta_1\ a_1\ a_2\ \gamma_1\ \gamma_2\ x\ y+z)$$

The above operators can be written in the form of the double series viz.,

$$(3.5)\quad \nabla_{x\ yz}(h) = \sum_{r=0}^{\infty}\sum_{s=0}^{\infty}\frac{(-\delta_1\ r+s)(-\delta_2\ r)(-\delta_3\ s)}{(1\ r)(1\ s)(h\ r+s)}$$

and

$$(3.6)\quad \Delta_{x\ yz}(h) = \sum_{r=0}^{\infty}\sum_{s=0}^{\infty}\frac{(-\delta_1\ r+s)(-\delta_2\ r)(-\delta_3\ s)}{(1\ r)(1\ s)(-h-\delta_1-\delta_2-\delta_3+1\ r+s)}$$

Type I

So as in article 2 from (3.5) and (3.1) we have

$$F_A(a_1\ a_1\ a_1\ \beta_1\ \beta_2, \beta_3\ \gamma_1\ \gamma_2, \gamma_3\ x\ y\ z)$$

$$= \sum_{r=0}^{\infty}\sum_{s=0}^{\infty}\frac{(-\delta_1\ r+s)(-\delta_2\ r)(-\delta_3\ s)}{(1\ r)(1\ s)(a_1\ r+s)}\times$$

$$\times F(a_1\ \beta_1\ \gamma_1\ x)\ F_2(a_1\ \beta_2, \beta_3\ \gamma_2, \gamma_3\ y\ z)$$

$$(3.7)\quad = \sum_{r=0}^{\infty}\sum_{s=0}^{\infty}\frac{(a_1\ r+s)(\beta_1\ r+s)(\beta_2\ r)(\beta_3\ s)}{(1\ r)(1\ s)(\gamma_1\ r+s)(\gamma_2\ r)(\gamma_3\ s)}x^{r+s}y^r z^s$$

$$\times F(a_1+r+s\ \beta_1+r+s\ \gamma_1+r+s\ x)\times$$

$$\times F_2(a_1+r+s, \beta_2+r\ \beta_3+s\ \gamma_2+r\ \gamma_3+s\ y\ z)$$

again using (3.6) in (3.2) we get

$$F(a_1\ \beta_1\ \gamma_1\ x)\ F_2(a_1\ \beta_2, \beta_3\ \gamma_2, \gamma_3\ y\ z)$$

$$= \sum_{r=0}^{\infty}\sum_{s=0}^{\infty}\frac{(-\delta_1\ r+s)(-\delta_2\ r)(-\delta_3\ s)}{(1\ r)(1\ s)(-a_1-\delta_1-\delta_2-\delta_3+1\ r+s)}\times$$

$$\times F_A(a_1\ a_1\ a_1\ \beta_1, \beta_2, \beta_3\ \gamma_1\ \gamma_2, \gamma_3\ x\ y\ z)$$

$$(3.8)\quad = \sum_{r=0}^{\infty}\sum_{s=0}^{\infty}(-)^{r+s}\frac{(a_1\ r+s)(\beta_1\ r+s)(\beta_2\ r)(\beta_3\ s)}{(1\ r)(1\ s)(\gamma_1\ r+s)(\gamma_2\ r)(\gamma_3\ s)}x^{r+s}y^r z^s$$

$$\times F_A(a_1+r+s\ a_1+r+s\ a_1+r+s\ \beta_1+r+s\ \beta_2+r\ \beta_3+s$$

$$\gamma_1+r+s\ \gamma_2+r\ \gamma_3+s\ x\ y\ z)$$

Type II

Now by using (3.6) and (3.5) in (3.3) and (3.4) respectively we get the following two expansions —

$$F_2(\beta_1\ a_1\ a_2\ \gamma_1\ \gamma_2\ x\ y+z)$$

$$(3.9)\quad = \sum_{r=0}^{\infty}\sum_{s=0}^{\infty}\frac{(a_1\ r)(\ \ r+2s)(\beta_1\ r+s)}{(1\ r)(1\ s)(\gamma_1\ r)(\gamma_2\ r+s)}x^r y^{r+s}z^s\times$$

$$\times F_M(\alpha_1+r, \alpha_2+r+2s, \alpha_2+r+2s, \beta_1+r+s, [\beta_1+r+s], \beta_1+r+s; \gamma_1+r, \gamma_2+r+2s, \gamma_2+r+2s; x, y, z)$$

and

$$F_M(\alpha_1, \alpha_2, \alpha_2, \beta_1, [\beta_1], \beta_1; \gamma_1, \gamma_2, \gamma_2; x, y, z)$$

$$(3.10) \quad = \sum_{r=0}^{\infty} \sum_{s=0}^{\infty} (-)^{r+s} \frac{(\alpha_1, r)(\alpha_2, r+2s)(\beta_1, r+s)}{(1, r)(1, s)(\gamma_1, r)(\gamma_2, r+2s)} x^r y^{r+s} z^s \times$$

$$\times F_2(\beta_1+r+s; \alpha_1+r, \alpha_2+r+2s; \gamma_1+r, \gamma_2+r+2s; x, y+z)$$

4 *Convergence Conditions*

To justify the proofs of our expansions it is necessary to prove the conditions of absolute convergence for these expansions. For this we take all the parameters real and positive and replace each term by their absolute value. We now like to know the bounds for the hypergeometric functions with positive variables when their positive parameters diverge to infinity in certain ways.

We establish these bounds by first proving the following simple inequalities which have been given in the form of lemmas.

( ) Lemmas I

$$(4.1) \quad F_2(\alpha_1+r+s; \beta_2+r, \beta_3+s; \gamma_2+r, \gamma_3+s; y, z)$$

$$< (1-y-z)^{-r-s} \frac{(\gamma_2, r)(\gamma_3, s)}{(\beta_2, r)(\beta_3, s)} F_2(\alpha_1; \beta_2, \beta_3; \gamma_2, \gamma_3; y, z)$$

$$(4.2) \quad F_2(\beta_1+r+s; \alpha_1+r, \alpha_2+r+2s; \gamma_1+r, \gamma_2+r+2s; x, y+z)$$

$$< (1-x-y-z)^{-r-s} \frac{(\gamma_1, r)(\gamma_2, r+2s)}{(\alpha_1, r)(\alpha_2, r+2s)} F_2(\beta_1; \alpha_1, \alpha_2; \gamma_1, \gamma_2; x, y+z)$$

$$(4.3) \quad F_K(\alpha_1+r, \alpha_2+r, \alpha_2+r, \beta_1+r, \beta_2, \beta_2+r; \gamma_1+r, \gamma_2, \gamma_2+r; x, y, z)$$

$$< (1-x-y-z)^{-r} \frac{(\gamma_1, r)(\gamma_2, r)}{(\alpha_1, r)(\beta_1, r)} F_K(\alpha_1, \alpha_2, \alpha_2, \beta_1, \beta_2, \beta_1; \gamma_1, \gamma_2, \gamma_2; x, y, z)$$

$$(4.4) \quad F_A(\alpha_1+r+s, \alpha_1+r+s, \alpha_1+s, \beta_1+r+s, \beta_2+r, \beta_3+s; \gamma_1+r+s, \gamma_2+r, \gamma_3+s; x, y, z)$$

$$< (1-x-y-z)^{-r-s} \frac{(\gamma_1, r+s)(\gamma_2, r)(\gamma_3, s)}{(\beta_1, r+s)(\beta_2, r)(\beta_3, s)} \times$$

$$\times F_A(\alpha_1, \alpha_1, \alpha_1, \beta_1, \beta_2, \beta_3; \gamma_1, \gamma_2, \gamma_3; x, y, z)$$

$$(4.5) \quad F_M(\alpha_1+r, \alpha_2+r+2s, \alpha_2+r+2s, \beta_1+r+s, \beta_1+r+s, \beta_1+r+s; \gamma_1+r, \gamma_2+r+2s, \gamma_2+r+2s; x, y, z)$$

$$< (1-y)^{-r-s} (1-x-z)^{-r-s} \frac{(\gamma_1, r)(\gamma_2, r+2s)}{(\alpha_1, r)(\alpha_2, r+2s)} \times$$

$$\times F_M(\alpha_1, \alpha_2, \alpha_2, \beta_1, \beta_1, \beta_1; \gamma_1, \gamma_2, \gamma_2; x, y, z)$$

(b) **Lemmas II**

(4 6) $F_N(\alpha_1+r\ \alpha_2+r\ \alpha_1, \beta_1+r\ \beta_2+r, \beta_1+r\ \ \gamma_1+r\ \gamma_2+r\ \gamma_3\ \ x\ y\ z)$

$$<(1-x)^{-r}\ (1-y)^{-r}\ \frac{(\gamma_1\ r)(\gamma_2, r)}{(\beta_1\ r)(\beta_2, r)}\times$$

$$\times F_N(\alpha_1\ \alpha_2\ \alpha_3\ \beta_1\ \beta_2, \beta_1\ \ \gamma_1\ \gamma_2, \gamma_3\ \ x\ y\ )$$

(4 7) $F_P(\alpha_1+r\ \alpha_1+r\ \alpha_2, \beta_1+r\ \beta_2+r\ \beta_1+r\ \ \gamma_1+r\ \gamma_2+r\ \gamma_1+r\ \ x, y\ )$

$$<(1-x-y)^{-r}\ \frac{(\gamma_1\ r)(\gamma_2\ r)}{(\beta_1, r)(\beta_2\ r)}\times$$

$$\times F_P(\alpha_1\ \alpha_1\ \alpha_2\ \beta_1\ \beta_2\ \beta_1\ \ \gamma_1\ \gamma_2, \gamma_2\ \ x\ y\ )$$

(4 8) $F_E(\alpha_1+2r\ \alpha_1+2r\ \alpha_1+2r\ \beta_1\ \beta_2+2r\ \beta_2+2r\ \ \gamma_1\ \gamma_2+2r\ \ x, y\ )$

$$<2^{2r}\ (1-y-z)^{-2r}\frac{(\gamma_2\ 2r)(\gamma_3\ 2r)}{(\alpha_1\ 2r)(\beta_2\ 2r)}\times$$

$$F_E(\alpha_1, \alpha_1\ \alpha_1\ \beta_1\ \beta_2\ \beta_2\ \ \gamma_1\ \gamma_2\ \gamma_3\ \ x\ y\ z)$$

5 (a) **Proofs of Lemmas in I**

To prove (4 1) we know that [1]

$$\frac{\Gamma(\beta_2+r)\ \Gamma(\beta_3+s)\ \Gamma(\gamma_2-\beta_2)\ \Gamma(\gamma_3-\beta_3)}{\Gamma(\gamma_2+r)\ \Gamma(\gamma_3+s)}\times$$

$$\times F_2(\alpha_1+r+s\ \ \beta_2+r\ \ \beta_3+s\ \ \gamma_2+r\ \ \gamma_3+s\ \ y\ )$$

$$=\int_0^1\int_0^1 u^{\beta_2+r-1}\, t^{\beta_3+s-1}\ (1-u)^{\gamma_2-\beta_2-1}(1-t)^{\gamma_3-\beta_3-1}\ y$$

$$(1-uy-t\ )^{-r-s-\alpha_1}\, du\, dt$$

$$=\int_0^1\int_0^1 u^{\beta_2-1}\ t^{\beta_3-1}\ (1-u)^{\gamma_2-\beta_2-1}\ \ (1-t)^{\gamma_3-\beta_3-1}\, y$$

$$\times(1-uy-t\ )^{-\alpha_1}\ \left[\frac{u^r\ \ t^s}{(1-uy-t\ )^{r+s}}\right] du\ dt$$

Now for r and s large and $0\leqslant u<1\ \ 0<t\leqslant 1$

$$\frac{u^r\ \ t^s}{(1-uy-t\ )^{r+s}}<\frac{1}{(1-y-\ )^{r+s}}$$

Thus

$$\frac{\Gamma(\beta_2)\ \Gamma(\beta_3)\ \Gamma(\gamma_2-\beta_2)\ \Gamma(\gamma_3-\beta_3)}{\Gamma(\gamma_2)\ \Gamma(\gamma_3)}\ \frac{(\beta_2, r)(\beta_3, s)}{(\gamma_2\ r)(\gamma_3\ s)}\times$$

$$\times F_2(\alpha_1+r+s\ \ \beta_2+r\ \ \beta_3+s\ \ \gamma_2+r\ \ \gamma_3+s\ \ y\ )$$

$$<(1-y-z)^{-r-s}\int_0^1\int_0^1 u^{\beta_2-1} v^{\beta_3-1}(1-u)^{\gamma_2-\beta_2-1}(1-v)^{\gamma_3-\beta_3-1}\times$$
$$\times(1-uy-vz)^{-\alpha_1}\,du\,dv$$

Now replacing the integral by its corresponding function F_p we get the inequality (4 1). The remaining other inequalities can be similarly proved.

(*b*) **Proofs of Lemmas in II**

To prove (4 6) we know that [4]

$$F_N(\alpha_1+r\ \alpha_2+r\ \alpha_3,\ \beta_1+r\ \beta_2+r\ \beta_1+r\ \ \gamma_1+r\ \gamma_2+r\ \gamma_3+r\ \ x\ y\ \ )$$
$$=\sum_{p=0}^{\infty}\frac{(\alpha_3,p)(\beta_1+r\ p)}{(1\ p)(\gamma_3+r\ p)}z^p\ F(\alpha_1+r\ \beta_1+r+p\ \ \gamma_1+r\ \ x)\times$$
$$\times F(\alpha_2+r\ \beta_2+r\ \ \gamma_2+r+p\ \ y)$$

using the inequality ([2] result 78)

$$(5.1)\quad F(a+r\ b+r\ \ c+r\ \ x)$$
$$<(1-x)^{-r}\frac{(c\ r)}{(b\ r)}F(a\ \ b\ \ c\ \ x)$$

we get

$$F_N(\ _1+r\ \alpha_2+r\ \alpha_3\ \beta_1+r\ \beta_2+r\ \beta_1+r\ \ \gamma\ +r\ \gamma_2+r\ \gamma\ +r\ \ x\ y\ z)$$
$$<(1-x)^{-r}\ (1-y)^{-r}\ \frac{(\gamma\ \ r)(\gamma_2,r)}{(\beta_1\ \ r)(\beta_2,r)}\sum_{p=0}^{\infty}\frac{(\alpha_3,p)(\beta_1\ \ p)}{(1\ \ p)(\gamma_3,p)}z^p\times$$
$$\times F(\alpha_1,\beta_1+p\ \ \gamma_1\ \ x)\ F(\alpha_2,\beta_2\ \ \gamma_2+p\ \ y)$$

hence the result.

To prove (4 7) we know that [4]

$$F_P(\alpha_1+r\ \alpha_1+r\ \alpha_2,\ \beta_1+r\ \beta_2+r\ \beta_1+r\ \gamma_1+r\ \gamma_2+r\ \gamma_3+r\ \ x\ y\ z)$$
$$=\sum_{p=0}^{\infty}\frac{(\alpha_2,p)(\beta_1+r\ p)}{(1\ p)\ (\gamma_3+r\ p)}z^p\ F_2(\alpha_1+r\ \beta_1+r+p\ \beta_2+r\ \ \gamma_1+r\ \gamma_2+r+p\ \ x\ y)$$

using the result ([2] result 83)

$$(5\ 2)\quad F_2(a+r\ b+r\ b'+r\ c+r\ c'+r\ \ x,y)$$
$$<(1-x-y)^{-r}\ \frac{(c\ r)(c'\ r)}{(b\ r)\ (b'\ r)}\ F_2(a,b,b'\ \ c,c'\ \ x\ y)$$

we get

$$F_P(\alpha_1+r\ \alpha_1+r\ \alpha_2,\ \beta_1+r\ \beta_2+r\ \beta_1+r\ \ \gamma_1+r\ \gamma\ +r\ \gamma_3+r\ \ x\ y\ z)$$
$$<(1-x-y)^{-r}\ \frac{(\gamma_1\ r)\ (\gamma_2,r)}{(\beta_1,r)\ (\beta_2,r)}\ \sum_{p=0}^{\infty}\frac{(\alpha_2\ \ p)\ (\beta_1\ \ p)}{(1\ \ p)\ \ (\gamma_3,p)}\ z^p\times$$
$$\times F_2(\alpha_1,\beta_1+p\ \ \beta_2;\gamma_1,\gamma_2+p\ \ x\ y)$$

hence the result.

Further to prove (4 8) we know that [4]

$$F_E(\alpha_1+2r,\ \alpha_1+2r,\ \alpha_1+2r,\ \beta_1,\ \beta_2+2r,\ \beta_2+2r,\ \gamma_1,\ \gamma_2+2r,\ \gamma_3+2r,\ x,\ y,\ z)$$

$$=\sum_{m=0}^{\infty} \frac{(\alpha_1+2r,\ m)\ (\beta_1,\ m)}{(1,\ m)\ (\gamma_1,\ m)}\ x^m \times$$

$$\times F_4(\alpha_1+2r+m,\ \beta_2+2r,\ \gamma_2+2r,\ \gamma_3+2r,\ y,\ z)$$

using the inequality ([2] result (86))

$$F_4(a+r,\ b+r,\ c+r,\ c'+r,\ x,\ y)$$

$$<2^{2r}(1-x-y)^{-2r}\frac{(c,\ r)(c',\ r)}{(a,\ r)(b,\ r)}F_4(a,\ b,\ c,\ c',\ x,\ y)$$

we have

$$F_E(\alpha_1+2r,\ \alpha_1+2r,\ \alpha_1+2r,\ \beta_1,\ \beta_2+2r,\ \beta_2+2r,\ \gamma_1,\ \gamma_2+2r,\ \gamma_3+2r,\ x,\ y,\ z)$$

$$<2^{2r}(1-y-z)^{-2r}\ \frac{(\gamma_2,\ 2r)\ (\gamma_3,\ 2r)}{(\alpha_1,\ 2r)\ (\beta_2,\ 2r)}\ \times$$

$$\times\sum_{m=0}^{\infty}\frac{(\alpha_1,\ m)(\beta_1,\ m)}{(1,\ m)\ (\gamma_1,\ m)}\ x^m\ F_4(\alpha_1+m,\ \beta_2,\ \gamma_2,\ \gamma_3,\ y,\ z)$$

hence the result.

We shall also make use of the following two inequalities given by ([2], result 78) and ([5] page 308) respectively viz.

(i) $F(\alpha_1+r,\ \beta_1+r,\ \gamma_1+r,\ x)$

$$<(1-x)^{-r}\ \frac{(\gamma_1,\ r)}{(\alpha_1,\ r)}\ F(\alpha_1,\ \beta_1,\ \gamma_1,\ x)$$

(ii) $F_2(\alpha_1+m,\ \beta_1,\ \beta_2+m,\ \gamma_1,\ \gamma_2+m,\ x,\ y)$

$$<(1-x-y)^{-m}\ \frac{(\gamma_2,\ m)}{(\alpha_1,\ m)}\ F_2(\alpha_1,\ \beta_1,\ \beta_2,\ \gamma_1,\ \gamma_2,\ x,\ y)$$

Using these asymptotic forms in our expansions we get the following regions of convergence —

$|x|+|y|+|\ \ |-|yz|<1$ in (2 4)

$|x|+|y|+|\ \ |+|xz|<1$ in (2.5)

$|x|+|y|<1$ in (2 6)

$|x|+|y|+|yz|<1$ in (2.7)

$|x|+|y|+|z|<1$ in (3 7) and (3 9)

$|x|-|y|+|\ \ |+|yz|+|x\ \ |<1$ in (3.8)

$|x|+|y|+|\ \ |+|xy|+|yz|<1$ in (3 10)

$|x|+|y|+|\ \ |+|\sqrt{yz}|<1$ in (2 8)

$|\sqrt{y}|+|\sqrt{z}|<1$ in (2 9)

We have also used the results 23 and 24 given by Burchnall and Chaundy [?] in our expansions.

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ON EXTENSORS*

HAR DUTT SINGH

Department of Mathematics B R. College Agra

The present work contains an investigation of the extensions and generalization of the extensions of a covariant differentiation process introduced by Craig[1] to obtain a tensor of one step higher covariant order in generalized Finsler spaces, and to study the behaviour of certain tensors and scalars in the light of each extended process. It also deals with the extensions of Bianchi and Veblen identities of a Riemannian manifold, by expressing them in terms of extensors in such a way that on reducing extensors to tensors the results obtained are in conformity with the established results for tensors. These identities have been also generalised in a space based on non-symmetric metric extensor and non-symmetric metric extended connection and by making use of the idea introduced by Einstein[2] and developed by Kaul and Mishra.[3] Certain theorems on reduced range extensors and on their contractions have been established and generalised.

I An Extension of a Covariant Differentiation Process

Considering tensors T^{α}_{β} whose components are functions of n variables given by x and their m derivatives x' x x'' $x^{(m)}$ Craig[1] obtained the covariant derivative

$$(1.1)\quad T^{\alpha}_{\beta} \quad {}_{x^{(m-1)\gamma}} - m\, T^{\alpha}_{\beta} \quad x^{(m)\lambda} \left\{ {\lambda \atop \gamma} \right\} \qquad (m \geqslant 2)$$

where

$$(1.2)\quad \left\{ {\lambda \atop \gamma} \right\} = x'^{\alpha}\, T^{\lambda}_{\gamma\alpha} + \tfrac{1}{2} x'^{\beta}\, f_{\gamma\delta\beta} \quad f^{\delta\lambda}$$

in which partial derivatives are indicated by subscripts and primes have been employed to denote differentiation with respect to the parameter Through out, a repeated letter in any term denotes a sum of n terms. The curves under the considerations are supposed to be given in parametric fo m.

We shall extend the process to obtain a tensor of one step higher covariant order by applying it to mixed tensors $T^{\alpha}_{\gamma}(x\ x\ \ x'')$ and $T^{\alpha}_{\gamma}(x\ x\ \ x\ \ x''\ \ x''')$ respectively First we take the tensor $T^{\alpha}_{\gamma}(x\ x\ \ x'')$ into consideration.

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The extended point transformation

(1.3) $x^{\alpha} = x^{\alpha}(y^1, y^2, \ldots, y^n)$, $x'^{\alpha} = \frac{\partial x^{\alpha}}{\partial y^i} y'^i$

$$x''^{\alpha} = \frac{\partial x^{\alpha}}{\partial y^i} y''^i + \frac{\partial^2 x^{\alpha}}{\partial y^i \partial y^j} y'^i y'^j$$

gives the following transformation in T^{α}_{γ}

(1.4) $\overline{T}^i_j (y, y', y'') = T^{\alpha}_{\gamma} (x, x', x'') \frac{\partial y^i}{\partial x^{\alpha}} \frac{\partial x^{\gamma}}{\partial y^j}$

in which y indicates n variables $y^1, y^2, y^3, \ldots, y^n$ and a similar notation is used for the derivatives y' and y''

Differentiating (1.4) with respect to y^k we get

(1.5) $\overline{T}^i_{j/k} = \left(T^{\alpha}_{\gamma/\beta} \frac{\partial x^{\beta}}{\partial y^k} + T^{\alpha}_{\gamma/\beta} \frac{\partial x'^{\beta}}{\partial y^k} + T^{\alpha}_{\gamma/\beta} \frac{\partial x''^{\beta}}{\partial y^k} \right) \frac{\partial y^i}{\partial x^{\alpha}} \frac{\partial x^{\gamma}}{\partial y^j}$

$$+ T^{\alpha}_{\gamma} \left(\frac{\partial^2 y^i}{\partial x^{\alpha} \partial x^{\beta}} \frac{\partial x^{\beta}}{\partial y^k} \frac{\partial x^{\gamma}}{\partial y^j} + \frac{\partial y^i}{\partial x^{\alpha}} \frac{\partial^2 x^{\gamma}}{\partial y^j \partial y^k} \right)$$

in which $\frac{\partial x'^{\beta}}{\partial y^k}$ are eliminated by

(1.6) $\overline{\left\{ {l \atop k} \right\}} \frac{\partial x^{\beta}}{\partial y^l} = \frac{\partial x'^{\beta}}{\partial y^k} + \left\{ {\beta \atop \delta} \right\} \frac{\partial x^{\delta}}{\partial y^k}$

a result due to J. H. Taylor[4]

To eliminate $\frac{\partial x''^{\beta}}{\partial y^k}$, we first write x''^{β} in the form

(1.7) $x''^{\beta} = \frac{\partial x^{\beta}}{\partial y^j} y''^j + \overline{T}^r_{jk} y'^j y'^k \frac{\partial x^{\beta}}{\partial y^r} - T^{\beta}_{\alpha\delta} x'^{\alpha} x'^{\delta}$

with the help of (1.2), (1.6) and $f_{\alpha\beta\gamma} x'^{\beta} = 0$ ([1], p. 248)

Differentiating the above equation with respect to y^k and using the tensor $T^{*} (x, x', x'') \overset{\text{def.}}{=} x''^{\beta} + T^{\beta}_{\alpha\delta} x'^{\alpha} x'^{\delta}$ and eliminating $\frac{\partial y''^j}{\partial y'^k}$ by means of the relationship [5]

(1.8) $\frac{\partial^2 x^{\beta}}{\partial y^j \partial y^k} = \overline{\Lambda}^l_{jk} \frac{\partial x^{\beta}}{\partial y^l} - \Lambda^{\beta}_{\alpha\delta} \frac{\partial x^{\alpha}}{\partial y^j} \frac{\partial x^{\delta}}{\partial y^k}$

where

$$(1.9) \quad \Lambda^{\beta}_{\alpha\delta} = T^{\beta}_{\alpha\delta} - \tfrac{1}{2} f^{\beta\gamma}\left(f_{\delta\gamma r}\left\{{}^{r}_{\alpha}\right\} + f_{\gamma\alpha r}\left\{{}^{r}_{\delta}\right\} - f_{\alpha\delta r}\left\{{}^{r}_{\gamma}\right\}\right)$$

we have

$$(1.10) \quad \frac{\partial x^{\beta}}{\partial y^{k}} = -\left|{}^{\beta}_{\gamma}\right| \frac{\partial x^{\gamma}}{\partial y^{k}} + \left|{}^{r}_{k}\right| \frac{\partial x^{\beta}}{\partial y^{r}} - 2\left\{{}^{\beta}_{\alpha}\right\}\left\{{}^{i}_{k}\right\}\frac{\partial x^{\alpha}}{\partial y^{i}} + 2\left\{{}^{r}_{i}\right\}\left\{{}^{i}_{k}\right\}\frac{\partial x^{\beta}}{\partial y^{r}}$$

where we have the non-tensor form

$$(1.11) \quad \left|{}^{\beta}_{\gamma}\right| \overset{\text{def.}}{=} T^{*\beta}_{x\gamma} - T^{*\beta}_{x'\alpha}\left\{{}^{\alpha}_{\gamma}\right\} + T^{*\alpha}\Lambda^{\beta}_{\alpha\gamma}$$

Substituting the values given by (1.6) (1.8) and (1.10) in (1.5) we get a new tensor of one higher covariant order

$$(1.12) \quad T^{\alpha}_{\gamma x\beta} - T^{\alpha}_{\gamma x'\delta}\left\{{}^{\delta}_{\beta}\right\} - T^{\alpha}_{\gamma x\delta}\left|{}^{\delta}_{\beta}\right| + T^{\delta}_{\gamma}\Lambda^{\alpha}_{\delta\beta} - T^{\alpha}_{\delta}\Lambda^{\delta}_{\gamma\beta}$$

where $\left\{{}^{\delta}_{\beta}\right\}$ $\left|{}^{\delta}_{\beta}\right|$ and $\Lambda^{\alpha}_{\delta\beta}$ are defined by (1.2) (1.11) and (1.9) respectively

2 *Properties*

(2.1) If we consider the tensor $T^{\alpha}(x\ x'\ x'')$ under the above process we get a tensor

$$T^{\alpha}_{x\beta} - T^{\alpha}_{x'\delta}\left\{{}^{\delta}_{\beta}\right\} - T^{\alpha}_{x\delta}\left|{}^{\delta}_{\beta}\right| + T^{\delta}\Lambda^{\alpha}_{\gamma\beta}$$

(2.2) If we take the tenso $T_{\gamma}(x\ x'\ x'')$ we have our new tensor

$$T_{\gamma x\beta} - T_{\gamma x'\delta}\left\{{}^{\delta}_{\beta}\right\} - T_{\gamma x\delta}\left|{}^{\delta}_{\beta}\right| - T_{\delta}\Lambda^{\delta}_{\gamma\beta}$$

(2.3) If we have a scalar $T(x, x'\ x'')$ the process yields a tensor

$$T_{x\beta} - T_{x'\delta}\left\{{}^{\delta}_{\beta}\right\} - T_{x\delta}\left|{}^{\delta}_{\beta}\right|$$

(2.4) If this process is performed on the tensor $T^{*\alpha}(x\ x'\ x'')$ the result is the zero tensor

(2.5) If the components of the tensor $T^{\alpha}_{\gamma}(x\ x'\ x'')$ do not contain x then (1.12) is reduced to the tensor

$$T^{\alpha}_{\gamma x\beta} - T^{\alpha}_{\gamma x'\delta}\left\{{}^{\delta}_{\beta}\right\} + T^{\delta}_{\gamma}\Lambda^{\alpha}_{\delta\beta} - T^{\alpha}_{\delta}\Lambda^{\delta}_{\gamma\beta}$$

(2.6) If the components of the tensor $T^{\alpha}_{\gamma}(x\ x\ x')$ do not contain x and x the result reduces to partial differentiation with respect to x.

(2.7) Evidently this process applies to a tensor of any rank and type and yields a tensor of one higher covariant rank than that of the original tensor.

(2.8) The rules for the derivatives of the sum (or difference) of the tensors of the same rank and kind and for the product of any tensors are conserved by the above process.

3 *Another Approach to the same Extension*

Now we shall see the existence of the extension of the above process in a different form. The general process will be shown clearly by using the tensor $T^{\alpha}_{\gamma}(x\ x\ x'\ x\ x''')$

The extended point transformation (1.3) gives the transformation of the tensor as

$$(3.1)\quad \overline{T}^{j}_{j}(y\ y\ y\ y\ y''')=T^{\alpha}_{\gamma}(x\ x'\ x\ x\ x''')\frac{\partial y^{i}}{\partial x^{\alpha}}\frac{\partial x^{\gamma}}{\partial y^{j}}$$

in which y as usual, represents n variables and a similar notation is used of the derivatives y y y and y'''

Differentiating (3.1) with respect to y^{k} and by the virtue of the relationships

$$\frac{\partial x^{\beta}}{\partial y^{k}}=\frac{\partial x^{\beta}}{\partial y^{k}}\quad \frac{\partial x^{\beta}}{\partial y^{k}}=3\frac{\partial x^{\beta}}{\partial y^{k}}\quad \frac{\partial x'''^{\beta}}{\partial y^{k}}=6\frac{\partial x^{\beta}}{\partial y^{k}}$$

which can be obtained, we get

$$(3.2)\quad \overline{T}^{i}_{jy^{k}}=\left(T^{\alpha}_{\gamma x}\,\beta\frac{\partial x^{\beta}}{\partial y^{k}}+3\,T^{\alpha}_{\gamma x'''\beta}\frac{\partial x^{\beta}}{\partial y^{k}}+6T^{\alpha}_{\gamma x}\,\beta\frac{\partial x^{\beta}}{\partial y^{k}}\right)\frac{\partial y^{i}}{\partial x^{\alpha}}\frac{\partial x^{\gamma}}{\partial y^{j}}$$

The derivatives $\frac{\partial x^{\beta}}{\partial y^{k}}$ and $\frac{\partial x^{\beta}}{\partial y^{k}}$ can be eliminated by means of (1.9) and (1.10) respectively. Consequently the new tensor whose covariant rank has been increased by one is

$$(3.3)\quad T^{\alpha}_{\gamma x}\,\beta-3\,T^{\alpha}_{\gamma x}\,\delta\left\{{\delta \atop \beta}\right\}-6\,T^{\alpha}_{\gamma x'''\delta}\left|{\delta \atop \beta}\right|$$

We can easily verify that the covariant rank of the tensor

$$T^{\alpha}_{\gamma} \quad {}_{x^{(m-2)\beta}} - (m-1)\, T^{\alpha}_{\gamma} \quad {}_{x^{(m-1)\delta}} \left\{ {\delta \atop \beta} \right\}$$
$$- \frac{m(m-1)}{2}\, T^{\alpha}_{\gamma} \quad {}_{x^{(m)\delta}} \left| {\delta \atop \beta} \right| \quad m \geq 4$$

is greater by one than that of the original tensor T^{α}_{γ} .. whose components are functions of x, x', x'', x''', x''''

This is the result which was obtained by Marie M. Johnson[8] taking m to be equal to 3 If the components of the tensor T^{α}_{γ} $(x \; x \; x''' \; x'''')$ do not contain the derivatives x then (3.3) reduces to Craig's covariant derivative. If there are no x''' and x then the result reduces to a partial differentiation with respect to x The other properties of the process can also be obtained in the light of some of those already stated.

4 The second Extension

We shall extend the above process to obtain a tensor of one higher covariant order The general process will be shown clearly by taking the tensor T^{α}_{γ} $(x \; x' \; x \; x''' \; x'''')$ into consideration The extended point transformation (1.3) gives the transformation of the tensor T^{α}_{γ} as expressed in (3 1)

Differentiating (3 1) with respect to y'^{k} and making use of the following general formulae

(4 1) $$\frac{\partial x^{(m-1)\beta}}{\partial y^{(m-2)k}} = (m-1)\frac{\partial x^{\beta}}{\partial y^{k}} \qquad \frac{\partial x^{(m)\beta}}{\partial y^{(m-2)k}} = \frac{m(m-1)}{2}\frac{\partial x^{\beta}}{\partial y^{k}}$$
$$\frac{\partial x^{(m+1)\beta}}{\partial y^{(m-2)k}} = \frac{(m+1)m(m-1)}{\lfloor 3}\frac{\partial x^{\beta}}{\partial y^{k}}$$

we obtain

(4.2) $$\bar{T}^{\alpha}_{j,k} = \left(T^{\alpha}_{\gamma x^{\prime}\beta}\frac{\partial x^{\beta}}{\partial y^{k}} + 2T^{\alpha}_{\gamma x^{\prime\prime}\beta}\frac{\partial x^{\beta}}{\partial y^{k}} + 3T^{\alpha}_{\gamma x^{\prime\prime\prime}\beta}\frac{\partial x^{\beta}}{\partial y^{k}} + 4T^{\alpha}_{\gamma x^{\prime\prime\prime\prime}\beta}\frac{\partial x^{\beta}}{\partial y^{k}} \right) \frac{\partial y^{i}}{\partial x^{\alpha}} \frac{\partial x^{\gamma}}{\partial y^{j}}$$

The quantities $\frac{\partial x^{\beta}}{\partial y^{k}}$ and $\frac{\partial x^{\prime\prime\beta}}{\partial y^{k}}$ are eliminated by means of (1 6) and (1.10) respectively

To eliminate $\frac{\partial x'^{\beta}}{\partial y^{k}}$ we first write x'^{β} in the form

$$(4\cdot3)\quad x'^{\beta} = \left(y''^{r} + \overline{T}^{*j}\left\{\overline{\begin{smallmatrix} r \\ j \end{smallmatrix}}\right\} + \overline{T}^{*r}_{y^{j}} y^{j} + \overline{T}^{*r}_{y'^{j}} y''^{j} \right) \frac{\partial x^{\beta}}{\partial y^{r}}$$
$$- \left(T^{*\alpha}\left\{\begin{smallmatrix} \beta \\ \alpha \end{smallmatrix}\right\} + T^{*\beta}_{x^{\gamma}} x'^{\gamma} + T^{*\beta}_{x'^{\gamma}} x''^{\gamma} \right)$$

by differentiating (1·7) with respect to the parameter and using the tensor (1·11)

By means of (1·6) (1·8) and (1·10) and using the tensor

$$(4\cdot4)\quad Q^{\beta}(x, x', x'', x''') \stackrel{\text{def.}}{=} x'''^{\beta} + T^{*\alpha}\left\{\begin{smallmatrix} \beta \\ \alpha \end{smallmatrix}\right\} + T^{*\beta}_{x^{\delta}} x'^{\delta} + T^{*\beta}_{x'^{\delta}} x''^{\delta}$$

the partial derivatives of (4·3) can be put in the form

$$(4\cdot5)\quad \frac{\partial x'^{\beta}}{\partial y^{k}} = -\left\|\begin{smallmatrix} \beta \\ \gamma \end{smallmatrix}\right\| \frac{\partial x^{\gamma}}{\partial y^{k}} + \left\|\overline{\begin{smallmatrix} r \\ k \end{smallmatrix}}\right\| \frac{\partial x^{\beta}}{\partial y^{r}}$$
$$+3\left[\left(\left|\overline{\begin{smallmatrix} l \\ k \end{smallmatrix}}\right| \left\{\overline{\begin{smallmatrix} r \\ l \end{smallmatrix}}\right\} + \left\{\overline{\begin{smallmatrix} l \\ k \end{smallmatrix}}\right\} \left|\overline{\begin{smallmatrix} r \\ l \end{smallmatrix}}\right| + 2\left\{\overline{\begin{smallmatrix} r \\ i \end{smallmatrix}}\right\}\left\{\overline{\begin{smallmatrix} i \\ l \end{smallmatrix}}\right\}\left\{\overline{\begin{smallmatrix} l \\ k \end{smallmatrix}}\right\} \right) \frac{\partial x^{\beta}}{\partial y^{r}}\right.$$
$$\left. - \left(\left|\overline{\begin{smallmatrix} l \\ k \end{smallmatrix}}\right| \left\{\begin{smallmatrix} \beta \\ \delta \end{smallmatrix}\right\} + \left\{\overline{\begin{smallmatrix} l \\ k \end{smallmatrix}}\right\} \left|\begin{smallmatrix} \beta \\ \delta \end{smallmatrix}\right| + 2\left\{\overline{\begin{smallmatrix} l \\ i \end{smallmatrix}}\right\}\left\{\overline{\begin{smallmatrix} i \\ k \end{smallmatrix}}\right\}\left\{\begin{smallmatrix} \beta \\ \delta \end{smallmatrix}\right\} \right) \frac{\partial x^{\delta}}{\partial y^{l}} \right]$$

in which we have the non tensor form

$$(4\cdot6)\quad \left\|\begin{smallmatrix} \beta \\ \gamma \end{smallmatrix}\right\| \stackrel{\text{def}}{=} Q^{\beta}_{x^{\gamma}} - Q^{\beta}_{x'^{\alpha}} \left\{\begin{smallmatrix} \alpha \\ \gamma \end{smallmatrix}\right\} - Q^{\beta}_{x''^{\alpha}} \left|\begin{smallmatrix} \alpha \\ \gamma \end{smallmatrix}\right| + Q^{\alpha} \Lambda^{\beta}_{\alpha\gamma}$$

Substituting the values given by (1·6) (1·10) and (4·5) in the equation (4·2) we have

$$(4\cdot7)\quad T^{\alpha}_{\gamma x''' \beta} - 2T^{\alpha}_{\gamma x'' \delta}\left\{\begin{smallmatrix} \delta \\ \beta \end{smallmatrix}\right\} - 3T^{\alpha}_{\gamma x''' \delta}\left|\begin{smallmatrix} \delta \\ \beta \end{smallmatrix}\right| - 4T^{\alpha}_{\gamma x'''' \delta}\left[\begin{smallmatrix} \delta \\ \beta \end{smallmatrix}\right]$$

as our new tensor

We can easily verify by virtue of the general relations in (4·1) that the covariant rank of the tensor

$$(4\cdot8)\quad T^{\alpha}_{\gamma\ldots x^{(m-3)\beta}} - (m-2)\, T^{\alpha}_{\gamma\ldots x^{(m-2)\delta}}\left\{\begin{smallmatrix} \delta \\ \beta \end{smallmatrix}\right\}$$
$$- \frac{(m-1)(m-2)}{2}\, T^{\alpha}_{\gamma\ldots x^{(m-1)\delta}}\left|\begin{smallmatrix} \delta \\ \beta \end{smallmatrix}\right| - \frac{m(m-1)(m-2)}{\lfloor 3}\, T^{\alpha}_{\gamma\ldots x^{(m)\delta}}\left[\begin{smallmatrix} \delta \\ \beta \end{smallmatrix}\right]$$

is one greater than that of the original tensor $T^{\alpha}_{\gamma\ldots}$ whose components are functions of $(x, x', x'', \ldots, x^{(m)})$

5. Properties

(5.1) If the components of the tensor do not contain x then (4.7) reduces to the result obtained by Marie M. Johnson[8]

(5.2) If the components of the tensor do not contain x' and x'' derivatives, then the result is Craig's covariant derivative.

(5.3) If there are no x x and x then the result is partial differentiation.

(5.4) The properties given in (2.8) are preserved by the above process.

(5.5) If $m=3$ a scalar T $(x\ x\ x\ x\ )$ will give rise to a covariant tensor when the tensor equations are differentiated with respect to y^k instead of y^k. The tensor so obtained is

$$T_{x^\beta} - T_{x^\delta}\left\{ {\delta \atop \beta} \right\} - T_{x'\delta} \left| {\delta \atop \beta} \right| - T_{x''\delta} \left\| {\delta \atop \beta} \right\|$$

(5.6) If $m=3$ a tensor T^{α}_{γ} $(x\ x\ x\ x\ )$ is used under the process (5.5) the new tensor of one higher covariant rank is

$$T^{\alpha}_{\gamma x^\beta} - T^{\alpha}_{\gamma x^\delta}\left\{ {\delta \atop \beta} \right\} - T^{\alpha}_{\gamma x'\delta} \left| {\delta \atop \beta} \right| - T^{\alpha}_{\gamma x''\delta} \left\| {\delta \atop \beta} \right\| + T^{\delta}_{\gamma} \wedge^{\alpha}_{\delta\beta} - T^{\alpha}_{\delta} \wedge^{\delta}_{\gamma\beta}$$

and so, the tensors T^{α} $(x\ x'\ x\ x''')$ and T_{γ} $(x\ x\ x\ x''')$ will give rise to the new tensors

$$T^{\alpha}_{x^\beta} - T^{\alpha}_{x^\delta}\left\{ {\delta \atop \beta} \right\} - T^{\alpha}_{x'\delta} \left| {\delta \atop \beta} \right| - T^{\alpha}_{x''\delta} \left\| {\delta \atop \beta} \right\| + T^{\delta} \wedge^{\alpha}_{\delta\beta}$$

and

$$T_{\gamma x^\beta} - T_{\gamma x^\delta}\left\{ {\delta \atop \beta} \right\} - T_{\gamma x'\delta} \left| {\delta \atop \beta} \right| - T_{\gamma x''\delta} \left\| {\delta \atop \beta} \right\| - T_{\delta} \wedge^{\delta}_{\gamma\beta}$$

respectively

We may note cursorily that the process indicated in (5.5) applies to a tensor of any type and rank and yields a tensor of one higher covariant order than that of original tensor

(5.7) Q^{α} $(x, x\ x\ x''')$ yields zero tensor under the process indicated in (5.5)

6. The Third Extension

We have already discussed the first and second extensions of a covariant differentiation process. We shall now consider the existence of further extension of the process for tensors whose components are functions of x x

$x, x \quad x \quad x'''$ We shall illustrate this process in the usual manner by taking the tensor $T^{\alpha}_{\gamma}(x\ x\ x, x\ , x'''\ x'')$ into consideration.

The extended point transformation (1.3) gives the following transformation in T^{α}_{γ}

$$(6.1)\quad \overline{T}^{i}_{j}(y\ y\ y\ y\ y''\ y'')=T^{\alpha}_{\gamma}(x\ x', x\ x\ x'', x''')\frac{\partial y^{i}}{\partial x^{\alpha}}\frac{\partial x^{\gamma}}{\partial y^{j}}$$

Differentiating the above equation with respect to y^{k} and making use of the general formulae (4.1) and

$$(6.2)\quad \frac{\partial x^{(m+2)\beta}}{\partial y^{(m-2)k}}=(m+2)(m+1)m(m-1)\frac{\partial x^{\beta}}{\lfloor 4\ \partial y^{k}}$$

we have

$$(6.3)\quad \overline{T}^{i}_{j\,y^{k}}=\Big(T^{\alpha}_{\gamma x^{\beta}}\frac{\partial x^{\beta}}{\partial y^{k}}+2T^{\alpha}_{\gamma x'^{\beta}}\frac{\partial x'^{\beta}}{\partial y^{k}}+3T^{\alpha}_{\gamma x''^{\beta}}\frac{\partial x''^{\beta}}{\partial y^{k}}$$

$$+4T^{\alpha}_{\gamma x'''^{\beta}}\frac{\partial x^{\beta}}{\partial y^{k}}+5T^{\alpha}_{\gamma x}\ {}_{\beta}\frac{\partial x^{\beta}}{\partial y^{k}}\Big)\frac{\partial y^{i}}{\partial x^{\alpha}}\frac{\partial x^{\gamma}}{\partial y^{j}}$$

The quantities $\frac{\partial x'^{\beta}}{\partial y^{k}}$ $\frac{\partial x^{\beta}}{\partial y^{k}}$ and $\frac{\partial x^{\beta}}{\partial y^{k}}$ are eliminated by means of (1.6) (1.10) and (4.5) respectively

To eliminate $\frac{\partial x'''^{\beta}}{\partial y^{k}}$ we first write $x\ ^{\beta}$ in the form

$$(6.4)\quad x'^{\beta}=\Big(y\ ^{i}+\overline{Q}^{j}\left\{{i \atop j}\right\}+\overline{Q}^{i}_{y^{j}}y^{j}+\overline{Q}^{i}_{y^{j}}y^{\prime j}+\overline{Q}^{i}_{y^{j}}y^{\prime\prime j}\Big)\frac{\partial x^{\beta}}{\partial y^{i}}$$

$$-\Big(Q^{\alpha}\left\{{\beta \atop \alpha}\right\}+Q^{\beta}_{x^{\gamma}}x^{\gamma}+Q^{\beta}_{x\ \gamma}x^{\gamma}+Q^{\beta}_{x\ \gamma}x''^{\gamma}\Big)$$

by differentiating (4.3) with respect to the parameter and using the result (4.4)

By means of formulae (1.6) (1.8) (1.10) and (4.5) and the tensor

$$(6.5)\quad R^{\beta}(x\ x\ x\ x\ x)\overset{\text{def}}{=\!=}x\ ^{\beta}+Q^{\alpha}\left\{{\beta \atop \alpha}\right\}+Q^{\beta}_{x\gamma}x^{\gamma}$$

$$+Q^{\beta}_{x'\gamma}x^{\gamma}+Q^{\beta}_{x\ \gamma}x''$$

the partial derivatives of (6.4) can be put in the form

$$(6.6)\quad \frac{\partial x'^{\beta}}{\partial y^{k}}=-\left\|{\beta \atop \gamma}\right\|\frac{\partial x^{\gamma}}{\partial y^{k}}+\left\|{i \atop jk}\right\|\frac{\partial x^{\beta}}{\partial y^{i}}+\Big(4\left|{i \atop k}\right|\left(\frac{i}{l}\right)+6\left|{i \atop l}\right|$$

$$+12\overline{\left\{ {r \atop i} \right\}}\,\overline{\left\{ {i \atop l} \right\}}\,\overline{\left| {l \atop k} \right|}+4\overline{\left\{ {l \atop k} \right\}}\,\overline{\left|\left\| {r \atop l} \right\|\right|}+12\overline{\left\{ {i \atop k} \right\}}\,\overline{\left| {i \atop l} \right|}\,\overline{\left\{ {r \atop i} \right\}}$$

$$+12\overline{\left\{ {l \atop k} \right\}}\,\overline{\left\{ {l \atop l} \right\}}\,\overline{\left| {r \atop i} \right|}+24\overline{\left\{ {l \atop k} \right\}}\,\overline{\left\{ {r \atop i} \right\}}\,\overline{\left\{ {i \atop i} \right\}}\,\overline{\left\{ {i \atop l} \right\}}\Big)\frac{\partial x^{\beta}}{\partial y^{r}}$$

$$-\Big(4\overline{\left|\left\| {l \atop k} \right\|\right|}\left\{ {\beta \atop \delta} \right\}+6\overline{\left| {l \atop k} \right|}\left\{ {\beta \atop \delta} \right\}+12\overline{\left| {r \atop k} \right|}\,\overline{\left\{ {l \atop r} \right\}}\left\{ {\beta \atop \delta} \right\}+4\overline{\left\{ {l \atop k} \right\}}\left|\left\| {\beta \atop \delta} \right\|\right|$$

$$+12\overline{\left\{ {i \atop k} \right\}}\,\overline{\left| {l \atop i} \right|}\left\{ {\beta \atop \delta} \right\}+12\overline{\left\{ {i \atop k} \right\}}\,\overline{\left\{ {l \atop i} \right\}}\left| {\beta \atop \delta} \right|+24\overline{\left\{ {i \atop k} \right\}}\,\overline{\left\{ {l \atop i} \right\}}\,\overline{\left\{ {i \atop l} \right\}}$$

$$\left\{ {\beta \atop \delta} \right\}\Big)\frac{\partial x^{\delta}}{\partial y^{l}}$$

in which we have the non tensor form

$$(6.7)\quad \left|\left\| {\beta \atop \gamma} \right\|\right| \overset{\text{def.}}{=} R^{\beta}_{x\gamma} - R^{\beta}_{x'\alpha}\left\{ {\alpha \atop \gamma} \right\} - R^{\beta}_{x''\alpha}\left| {\alpha \atop \gamma} \right| - R^{\beta}_{x}{}_{\alpha}\left\| {\alpha \atop \gamma} \right\| + R^{\alpha}\Lambda^{\beta}_{\alpha\gamma}$$

Substituting the values given by (1·6) (1 10) (4 5) and (6·6) in the equation (6·3) we have a new tensor of one higher covariant rank

$$(6{\cdot}8)\quad T^{\alpha}_{\gamma x\,\beta} - 2T^{\alpha}_{\gamma x'\delta}\left\{ {\delta \atop \beta} \right\} - 3T^{\alpha}_{\gamma x''\delta}\left| {\delta \atop \beta} \right| - 4T^{\alpha}_{\gamma x'''\delta}\left\| {\delta \atop \beta} \right\|$$

$$-5T^{\alpha}_{\gamma x''''\delta}\left|\left\| {\delta \atop \beta} \right\|\right|$$

Because of the general relations (4 1) and (6·2) it is easy to verify that the covariant rank of the tensor

$$(6{\cdot}9)\quad T^{\alpha}_{\gamma\;\; x^{(m-4)}\beta} - (m-3)T^{\alpha}_{\gamma\;\; x^{(m-3)}\delta}\left\{ {\delta \atop \beta} \right\}$$

$$-\frac{(m-2)(m-3)}{2}T^{\alpha}_{\gamma\;\; x^{(m-2)}\delta}\left| {\delta \atop \beta} \right| - \frac{(m-1)(m-2)(m-3)}{\lfloor\underline{3}}T^{\alpha}_{\gamma\;\; x^{(m-1)}\delta}\left\| {\delta \atop \beta} \right\|$$

$$-\frac{m(m-1)(m-2)(m-3)}{\lfloor\underline{4}}T^{\alpha}_{\gamma\;\; x^{(m)}\delta}\left|\left\| {\delta \atop \beta} \right\|\right| \quad m \geq 3$$

is greater by one than that of the original tensor T^{α}_{γ} whose components are functions of x x' x $x^{(m)}$

The properties of the above process can be established in the light of those stated in section 5

7 A Generalization of the Extensors

By virtue of the first second and third extensions of covariant differentiation process we establish a generalization of the extensions of the process for tensors whose components contain derivatives of any order by making use of mathematical induction Thus, if the components T^{α}_{γ} of a given tensor are functions of x x $x^{(m)}$ then the quantities

$$T^{\alpha}_{\gamma} \;\; x^{(m-r+1)\beta} - (m-r+2)\; T^{\alpha}_{\gamma} \;\; x^{(m-r+2)\delta} \left\{ {\delta \atop \beta} \right\}$$

$$- \frac{(m-r+3)(m-r+2)}{2}\; T^{\alpha}_{\gamma} \;\; x^{(m-r+3)\delta} \left| {\delta \atop \beta} \right|$$

$$- \frac{(m-r+4)(m-r+3)(m-r+2)}{\lfloor 3}\; T^{\alpha}_{\gamma} \;\; x^{(m-r+4)\delta} \left\{ {\delta \atop \beta} \right\}$$

$$- \frac{(m-r+5)(m-r+4)(m-r+3)(m-r+2)}{\lfloor 4}$$

$$T^{\alpha}_{\gamma} \;\; x^{(m-r+5)\delta} \left\{ {\delta \atop \beta} \right\}$$

$$- \frac{m(m-1)(m-2) \quad (m-r+2)}{\lfloor r-1}\; T^{\alpha}_{\gamma} \;\; x^{(m)\delta} \left\| {\delta \atop \beta} \right\|$$

are the components of a tensor whose covariant order is greater by one than that of the original tensor T^{α}_{γ} The number of bars in the last term is $r-2$, and its value can be obtained by virtue of the relations (1 11) (4 6) (6 7)

Obviously this process yields properties similar to those stated above

8 Extended Bianchi and Veblen Identities

In dealing with extensors, we shall use only one root letter x for coordinate systems, and distinguish among different coordinate systems by means of letters employed as indices. Thus instead of denoting different coordinate systems by various letters of alphabet x, y etc., we shall denote them by x^i and x^r etc. where i j k and r s, t refer to first and second etc coordinate systems The derivatives with respect to parameter t of a parameterized arc will be denoted by means of Greek indices as superscript and partial derivatives with respect to x will be means of Greek subscripts viz.

$$x^{\prime\alpha} = \frac{dx^{\alpha}}{dt} \quad x^{(a)r} = \frac{d^{a} x^{r}}{dt^{a}} \quad F_{(\;)r} = \frac{\partial F}{\partial x^{(a)r}}$$

$$X^r_i = \frac{\partial x^r}{\partial x^i} \quad X^{(\alpha)i}_{(\beta)r} = \frac{\partial x^{(\alpha)i}}{\partial x^{(\beta)r}} \quad X^{r(\rho)}_{i} = \frac{d^\rho X^r_i}{dt^\rho}$$

Repeated lower case Latin indices indicate summation from one to n while repeated lower case Greek indices from O to M unless the contrary is indicated by the presence of summation signs giving the ranges. It is also necessary to assume that the curves under the discussion are of class c^M

The extensors, introduced first by Craig[6] appears as an extension of the tensor concept, and hold the tensor member as a part of its components From this fact Mme. Katsurada[7] introduced the excovariant differentiation with respect to the extended connection parameter $\Gamma^{\alpha i}_{\beta j\,\gamma k}$ of the *Mth* order derived from the connection parameter Γ^i_{jk} of an n-dimensional space. The extended connection parameter $\Gamma^{\alpha i}_{\beta j \gamma k}$ is given by

$$\Gamma^{\alpha i}_{\beta j\,\gamma k} = \binom{A}{BC}\, \Gamma^{i(A-B-C)}_{jk} \quad A=\alpha,\ B=\beta\ \ C=\gamma$$

where

$$\binom{A}{BC} = \frac{\lfloor A}{\lfloor B \lfloor C \lfloor A-B-C} \qquad A \geqslant B+C$$
$$= O \qquad\qquad A < B+C$$

With the help of this extended parameter and excovariant differentiation, the extended Ricci formulae for $V^{(\alpha)i}$ and $V_{(\alpha)i}$ may be stated as

$$(8\cdot 1)\quad V^{(\alpha)i}_{\ \ \ \beta j\ \ \gamma k} - V^{(\alpha)i}_{\ \ \ \gamma k\ \ \beta j} = V^{(\delta)l} R^{\alpha i}_{\delta l\, \beta j\, \gamma k}$$

and

$$(8\cdot 2)\quad V_{(\alpha)i\ \ \beta j\ \ \gamma k} - V_{(\alpha)i\ \ \gamma k\ \ \beta j} = -V_{(\delta)l} R^{\delta l}_{\alpha i\, \beta j\, \gamma k},$$

where

$$(8\cdot 3)\quad R^{\alpha i}_{\beta j \gamma k\, \delta l} = \Gamma^{\alpha i}_{\beta j\, \gamma k,\ \delta l} - \Gamma^{\alpha i}_{\beta j\, \delta l\ \ \gamma k} + \Gamma^{\alpha i}_{\rho k\, \delta l}\, \Gamma^{\rho h}_{\beta j\, \gamma k} - \Gamma^{\alpha i}_{\rho k\, \gamma k}\, \Gamma^{\rho h}_{\beta j\, \delta l}$$

and $|$ and denote excovariant and partial differentiation respectively We see that $R^{\alpha i}_{\beta j \gamma k \delta l}$ is a mixed extensor of the fourth order and shall call

it the curvature extensor or the extended Riemannian symbol of the second kind It satisfies the relations

$$(8.4)\quad R^{\alpha i}_{\beta j\gamma k\delta l}+R^{\alpha i}_{\beta j\delta l\gamma k}=0$$

$$(8.5)\quad R^{\alpha i}_{\beta j\gamma k\delta l}+R^{\alpha i}_{\gamma k\delta l\beta j}+R^{\alpha i}_{\delta l\beta j\gamma k}=0.$$

$$(8.6)\quad R^{\alpha i}_{\beta j\gamma k\delta l}=\binom{A}{BCD}R^{i}_{jkl}(A-B-C-D)\quad A=\alpha,\ B=\beta\ \ C=\gamma$$

$D=\delta$ where R^{i}_{jkl} is the curvature tensor in the Riemannian space

Differentiating $R^{\alpha i}_{\beta j\gamma k\delta l}$ excovariantly with respect to $x^{(\epsilon)m}$ we have

$$R^{\alpha i}_{\beta j\gamma k\delta l\ \epsilon m}=R^{\alpha i}_{\beta j\gamma k\delta l\ \epsilon m}+R^{\lambda h}_{\beta j\gamma k\delta l}\Gamma^{\alpha i}_{\lambda h\ \epsilon m}-R^{\alpha i}_{\lambda h\gamma k\delta l}\Gamma^{\lambda h}_{\beta j\epsilon m}$$

$$-R^{\alpha i}_{\beta j\lambda h\delta l}\Gamma^{\lambda h}_{\gamma k\ \epsilon m}-R^{\alpha i}_{\beta j\gamma k\lambda h}\Gamma^{\lambda h}_{\delta l\ \epsilon m}$$

Two similar equations are obtained by cyclic permutation of the indices γk, δl and ϵm. If the three equations are added we see that six terms of the second member cancel themselves by virtue of (8.4) and after substitut-ing the value of $R^{\alpha i}_{\beta j\gamma k\delta l}$ in the remaining terms, we find that all the second and first order derivative terms and the terms containing no derivatives of the symmetric extended connection of the space cancel themselves showing that

$$(8.7)\quad R^{\alpha i}_{\beta j\gamma k\delta l\ \epsilon m}+R^{\alpha i}_{\beta j\delta l\epsilon m\ \gamma k}+R^{\alpha i}_{\beta j\epsilon m\gamma k\ \delta l}=0$$

which is the extended Bianchi identity

If the four equations for $R^{\alpha i}_{\beta j\gamma k\delta l\ \epsilon m}$, $R^{\alpha i}_{\delta l\beta j\epsilon m\ \gamma k}$ $R^{\alpha i}_{\epsilon m\delta l\gamma k\ \beta j}$ and $R^{\alpha i}_{\gamma k\epsilon m\beta j\ \delta l}$ are added we find that the four terms of the second member cancel themselves by virtue of (8.4) and the remaining eight terms with -ive signs reduce themselves to four terms with +ive signs. Now substitut-ing the values $R^{\alpha i}_{\beta j\gamma k\delta l}$ in all the terms we find that all the second and first order partial derivatives terms and the terms containing no derivatives of the symmetric extended connection of the space cancel themselves showing that

$$(8.8)\quad R^{\alpha i}_{\beta j\ \gamma k\ \delta l\ \epsilon m}+R^{\alpha i}_{\delta l\ \beta j\ \epsilon m\ \gamma k}+R^{\alpha i}_{\epsilon m\delta l\gamma k\ \beta j}+R^{\alpha i}_{\gamma k\epsilon m\ \beta j\ \delta l}=0$$

which is the extended Veblen Indentity

It is interesting to note that by means of the theorem (8.6) the identities given by (8.7) and (8.8) directly follow because they have the same contents as the Bianchi and Veblen identities in essentials. However in the next development which follows in generalizations of these identities in a space based on non-symmetric metric extensor and non symmetric extended connection, we shall consider the cancellation of the terms in the light of (8.7) and (8.8)

9 Two Kinds of Excovariant Derivatives

We consider an n-dimensional space which is based on non-symmetric extensor $g_{\alpha i \beta j}$ $\left(g_{\alpha i \beta j} \neq g_{\beta j \alpha i} \right)$ and non-symmetric extended connection $\Gamma^{\alpha i}_{\beta j \gamma k}$ $\left(\Gamma^{\alpha i}_{\beta j \gamma k} \neq \Gamma^{\alpha i}_{\gamma k \beta j} \right)$ The connection $\Gamma^{\alpha i}_{\beta j \gamma k}$ can be written as a sum of its symmetric and skew-symmetric parts given by $\Gamma^{\alpha i}_{\underline{\beta j \gamma k}}$ and $\Gamma^{\alpha i}_{\underset{\vee}{\beta j \gamma k}}$ respectively

It is expressed as

(9.1) $\Gamma^{\alpha i}_{\beta j \gamma k} = \Gamma^{\alpha i}_{\underline{\beta j \gamma k}} + \Gamma^{\alpha i}_{\underset{\vee}{\beta j \gamma k}}$

Let us introduce a connection $\tilde{\Gamma}^{\alpha i}_{\beta j \gamma k}$ defined by

(9.2) $\tilde{\Gamma}^{\alpha i}_{\beta j \gamma k} = \Gamma^{\alpha i}_{\underline{\beta j \gamma k}} - \Gamma^{\alpha i}_{\underset{\vee}{\beta j \gamma k}}$

then from (9.1) and (9.2) we have

(9.3) $\tilde{\Gamma}^{\alpha i}_{\beta j \gamma k} = \Gamma^{\alpha i}_{\gamma k \beta j}$

Now we can define the excovariant derivative of an excontravariant exvector $U^{\alpha i}$ in the following two ways

(9.4) $U^{\alpha i}_{\underset{+}{;}\beta j} = U^{\alpha i}_{,\beta j} + U^{\gamma k} \, \Gamma^{\alpha i}_{\gamma k \beta j}$

and

(9.5) $U^{\alpha i}_{\underset{-}{;}\beta j} = U^{\alpha i}_{,\beta j} + U^{\gamma k} \, \tilde{\Gamma}^{\alpha i}_{\gamma k \beta j}$

where a comma (,) followed by doublet indices, denotes partial derivative, a (+) and a (−) sign below doublet indices followed by a semi-colon, indicates that an excovariant derivative has been formed with respect to the connection

$\Gamma^{\alpha i}_{\beta j\ \gamma k}$ and $\overset{\smile}{\Gamma}{}^{\alpha i}_{\beta j\ \gamma k}$ respectively as far as that pair of indices is concerned.

By virtue of (9 3) the equation (9.5) can be written also in the form

$$(9\ 6)\quad U^{\alpha i}_{\overline{\beta j}} = U^{\alpha i}_{\beta j} + U^{\gamma k}\ \Gamma^{\alpha i}_{\beta j\ \gamma k}$$

This duality in the nature of excovariant derivative yields two curvature extensors given as (8.3) and

$$(9\ 7)\quad \overset{\smile}{R}{}^{\alpha i}_{\beta j\ \gamma k\ \delta l} = \Gamma^{\alpha i}_{\gamma k\ \beta j\ \ \delta l} - \Gamma^{\alpha i}_{\delta l\ \beta j\ \ \gamma k} + \Gamma^{\alpha l}_{\delta l\ \rho k}\ \Gamma^{\rho k}_{\gamma k\ \beta j} - \Gamma^{\alpha i}_{\gamma k\ \rho k}\ \Gamma^{\rho k}_{\delta l\ \beta j}$$

We shall find identities in this space corresponding to extended Bianchi and Veblen identities.

10 Generalizations of Bianchi Identities

In extended Bianchi identities (8 7) we find that six second order partial derivatives of the symmetric extended connection of the space cancel themselves. These terms also vanish in the case of non-symmetric extended connection Therefore on the left hand side of our identities we have only three terms as before. But a complication arises due to the fact that in this case some of the thirtysix—first order derivatives of the extended connection do not cancel as they do in the case of symmetric extended connection. However if we confine ourselves to the excovariant diff rentiation with respect to the excontravariant and first excovariant indices only we may state the fundamental identity as

$$(10\ 1)\quad R^{\alpha i}_{\underset{+}{\beta j}\ \gamma k\ \delta l\ \ \epsilon m} + R^{\alpha i}_{\underset{+}{\beta j}\ \delta l\ \epsilon m\ \ \gamma k} + R^{\alpha i}_{\underset{+}{\beta j}\ \epsilon m\ \gamma k\ \ \delta l} = 0.$$

In order that the identity may be in extensorial form, it is necessary to put some sign +ve or —ve below the second and the third excovariant indices of the terms in the above equation It can be verified that by putting opposite signs under the pair of indices γk and ϵm of the first and δl of the term δl and ϵm of the first and the third term and δl and γl of the second and the third term the extra terms introduced cancel themselves. The possible number of ways in which this selection of +ve and — can be made are eight. But out of eight identities, thus obtained there are only four independent indent ties which are written as

$$(10\text{·}2)\quad R^{\alpha i}_{\underset{+}{\beta j}\ \underset{+}{\gamma k}\ \underset{+}{\delta l}\ \ \epsilon m} + R^{\alpha i}_{\underset{+}{\beta j}\ \underset{+}{\delta l}\ \underset{-}{\epsilon m}\ \ \gamma k} + \Gamma^{\alpha i}_{\underset{+}{\beta j}\ \underset{-}{\epsilon m}\ \underset{-}{\gamma k}\ \ \delta l} = 0$$

$$(10\cdot3)\quad \underset{\underset{+}{\beta j}\ \underset{+}{\gamma k}\ \underset{+}{\delta l}\ \epsilon m}{\overset{\alpha i}{R^{+}}} + \underset{\underset{+}{\beta j}\ \underline{\delta l}\ \underline{\epsilon m}\ \gamma k}{\overset{\alpha i}{R^{+}}} + \underset{\underset{+}{\beta j}\ \underline{\epsilon m}\ \underset{+}{\gamma k}\ \delta l}{\overset{\alpha i}{R^{+}}} = 0$$

$$(10\cdot4)\quad \underset{\underset{+}{\beta j}\ \underset{+}{\gamma k}\ \underline{\delta l}\ \epsilon m}{\overset{\alpha i}{R^{+}}} + \underset{\underset{+}{\beta j}\ \underset{+}{\delta l}\ \underline{\epsilon m}\ \gamma k}{\overset{\alpha i}{R^{+}}} + \underset{\underset{+}{\beta j}\ \underset{+}{\epsilon m}\ \underline{\gamma k}\ \delta l}{\overset{\alpha i}{R^{+}}} = 0$$

$$(10\cdot5)\quad \underset{\underset{+}{\beta j}\ \underset{-}{\gamma k}\ \underset{+}{\delta l}\ \epsilon m}{\overset{\alpha i}{R^{+}}} + \underset{\underset{+}{\beta j}\ \underline{\delta l}\ \underset{+}{\epsilon m}\ \gamma k}{\overset{\alpha i}{R^{+}}} + \underset{\underset{+}{\beta j}\ \underline{\epsilon m}\ \underset{+}{\gamma k}\ \delta l}{\overset{\alpha i}{R^{+}}} = 0$$

11 Generalizations of Veblen Identities

In extended Veblen identities (8·8) we find that eight second order partial derivatives of the symmetric extended connection of the space cancel themselves but in the case of non-symmetric extended connection these terms do not. However if we add more terms

$\Gamma^{\alpha i}_{\gamma k\, \beta j\ \delta l\, \beta j} - \Gamma^{\alpha i}_{\delta l\, \beta j\ \gamma k\ \epsilon m}$ introduced by $\overset{\smile}{R}{}^{\alpha i}_{\delta l\, \beta j\, \epsilon m\ \gamma k}$ and other such terms introduced by $\overset{\smile}{R}{}^{\alpha i}_{\delta l\, \beta j\, \epsilon m\ \gamma k}$, $\overset{\smile}{R}{}^{\alpha i}_{\epsilon m\, \delta l\, \gamma k\ \beta j}$ and $\overset{\smile}{R}{}^{\alpha i}_{\gamma k\, \epsilon m\, \beta j\ \delta l}$ to the second order partial derivatives of (8·8) we see that the result equals zero. Consequently we shall have on the left hand side of our identities eight terms instead of four

If we confine ourselves to the excovariant differentiation with respect to the excontravariant and the second and third excovariant indices only we shall have our fundamental identity as

(11·1)
$$\overset{\alpha i}{R^{-}}_{\beta j\, \underline{\gamma k}\, \underline{\delta l}\ \epsilon m} + \overset{\alpha i}{R^{-}}_{\delta l\, \underline{\beta j}\, \underline{\epsilon m}\ \gamma k} + \overset{\alpha i}{R^{-}}_{\epsilon m\, \underline{\delta l}\, \underline{\gamma k}\ \beta j} + \overset{\alpha i}{R^{-}}_{\gamma k\, \underline{\epsilon m}\, \underline{\beta j}\ \delta l}$$
$$+ \underset{\beta j\ \underset{+}{\gamma k}\ \underset{+}{\delta l}\ \epsilon m}{\overset{\smile\,\alpha i}{R^{+}}} + \underset{\delta l\ \underset{+}{\beta j}\ \underset{+}{\epsilon m}\ \gamma k}{\overset{\smile\,\alpha i}{R^{+}}} + \underset{\epsilon m\ \underset{+}{\delta l}\ \underset{+}{\gamma k}\ \beta j}{\overset{\smile\,\alpha i}{R^{+}}} + \underset{\gamma k\ \underset{+}{\epsilon m}\ \underset{+}{\beta j}\ \delta l}{\overset{\alpha i}{R^{+}}} = 0$$

It is necessary to put some sign +ve or −ve under the first excovariant indices of the terms in the above equation in order to get the identity in extensorial form. It can be seen that by putting opposite signs under the pairs of first excovariant indices βj ϵm and δl γk in the left members of (11·1) the extra terms cancel themselves.

Obviously the possible number of ways for the selection of +ve and −ve signs are sixteen, but out of sixteen identities, thus obtained, we get

only four independent identities which are

$$\text{(11.2)}\quad \overset{-}{R}{}^{\alpha i}_{\underset{-}{\beta j}\,\underset{-}{\gamma k}\,\underset{-}{\delta l}\;\epsilon m} + \overset{-}{R}{}^{\alpha i}_{\underset{-}{\delta l}\,\underset{-}{\beta j}\;\underset{-}{\epsilon m}\;\gamma k} + \overset{-}{R}{}^{\alpha i}_{\underset{+}{\epsilon m}\,\underset{-}{\delta l}\,\underset{-}{\gamma k}\;\beta j}$$

$$+ \overset{-}{R}{}^{\alpha i}_{\underset{+}{\gamma k}\;\underset{-}{\epsilon m}\,\underset{-}{\beta j}\;\delta l} + \overset{+}{R}{}^{\alpha i}_{\underset{+}{\beta j}\,\underset{+}{\gamma k}\,\underset{+}{\delta l}\;\epsilon m} + \overset{+}{R}{}^{\alpha i}_{\underset{+}{\delta l}\,\underset{+}{\beta j}\;\underset{+}{\epsilon m}\;\gamma k} + \overset{+}{R}{}^{\alpha i}_{\underset{-}{\epsilon m}\,\underset{+}{\delta l}\,\underset{+}{\gamma k}\;\beta j}$$

$$+ \overset{-}{R}{}^{\alpha i}_{\underset{-}{\gamma k}\;\underset{+}{\epsilon m}\,\underset{+}{\beta j}\;\delta l} = 0$$

$$\text{(11.3)}\quad \overset{-}{R}{}^{\alpha i}_{\underset{-}{\beta j}\,\underset{-}{\gamma k}\,\underset{-}{\delta l}\;\epsilon m} + \overset{-}{R}{}^{\alpha i}_{\underset{-}{\delta l}\,\underset{-}{\beta j}\;\underset{-}{\epsilon m}\;\gamma k} + \overset{-}{R}{}^{\alpha i}_{\underset{+}{\epsilon m}\,\underset{-}{\delta l}\,\underset{-}{\gamma k}\;\beta j}$$

$$+ \overset{-}{R}{}^{\alpha i}_{\underset{+}{\gamma k}\;\underset{-}{\epsilon m}\,\underset{-}{\beta j}\;\delta l} + \overset{+}{R}{}^{\alpha i}_{\underset{-}{\beta j}\,\underset{+}{\gamma k}\,\underset{-}{\delta l}\;\epsilon m} + \overset{+}{R}{}^{\alpha i}_{\underset{-}{\delta l}\,\underset{+}{\beta j}\;\underset{+}{\epsilon m}\;\gamma k} + \overset{+}{R}{}^{\alpha i}_{\underset{+}{\epsilon m}\,\underset{+}{\delta l}\,\underset{+}{\gamma k}\;\beta j}$$

$$+ \overset{+}{R}{}^{\alpha i}_{\underset{+}{\gamma k}\;\underset{+}{\epsilon m}\,\beta j\;\delta l} = 0$$

$$\text{(11.4)}\quad \overset{-}{R}{}^{\alpha i}_{\underset{-}{\beta j}\,\underset{-}{\gamma k}\,\underset{-}{\delta l}\;\epsilon m} + \overset{-}{R}{}^{\alpha i}_{\underset{-}{\delta l}\,\underset{-}{\beta j}\;\underset{-}{\epsilon m}\;\gamma k} + \overset{-}{R}{}^{\alpha i}_{\underset{+}{\epsilon m}\,\underset{-}{\delta l}\,\underset{-}{\gamma k}\;\beta j}$$

$$+ \overset{-}{R}{}^{\alpha i}_{\underset{+}{\gamma k}\;\underset{-}{\epsilon m}\,\underset{-}{\beta j}\;\delta l} + \overset{+}{R}{}^{\alpha i}_{\underset{+}{\beta j}\,\underset{+}{\gamma k}\,\underset{+}{\delta l}\;\epsilon m} + \overset{+}{R}{}^{\alpha i}_{\underset{-}{\delta l}\,\underset{+}{\beta j}\;\underset{+}{\epsilon m}\;\gamma k} + \overset{+}{R}{}^{\alpha i}_{\underset{-}{\epsilon m}\,\underset{+}{\delta l}\,\underset{+}{\gamma k}\;\beta j}$$

$$+ \overset{+}{R}{}^{\alpha i}_{\underset{+}{\gamma k}\;\underset{+}{\epsilon m}\,\beta j\;\delta l} = 0$$

$$\text{(11.5)}\quad \overset{-}{R}{}^{\alpha i}_{\underset{-}{\beta j}\,\underset{-}{\gamma k}\,\underset{-}{\delta l}\;\epsilon m} + \overset{-}{R}{}^{\alpha i}_{\underset{-}{\delta l}\,\underset{-}{\beta j}\;\underset{-}{\epsilon m}\;\gamma k} + \overset{-}{R}{}^{\alpha i}_{\underset{+}{\epsilon m}\,\underset{-}{\delta l}\,\underset{-}{\gamma k}\;\beta j}$$

$$+ \overset{-}{R}{}^{\alpha i}_{\underset{+}{\gamma k}\;\underset{-}{\epsilon m}\,\underset{-}{\beta j}\;\delta l} + \overset{+}{R}{}^{\alpha i}_{\underset{-}{\beta j}\,\underset{+}{\gamma k}\,\underset{+}{\delta l}\;\epsilon m} + \overset{+}{R}{}^{\alpha i}_{\underset{+}{\delta l}\,\underset{+}{\beta j}\;\underset{+}{\epsilon m}\;\gamma k} + \overset{+}{R}{}^{\alpha i}_{\underset{+}{\epsilon m}\,\underset{+}{\delta l}\,\underset{+}{\gamma k}\;\beta j}$$

$$+ \overset{-}{R}{}^{\alpha i}_{\underset{-}{\gamma k}\;\underset{+}{\epsilon m}\,\beta j\;\delta l} = 0$$

12 Contraction of Extensors

The theories of extensors of reduced range and those of contractions over full and reduced range, have been treated by Craig[4, 5] and A. Kawaguchi[9]. Here we shall concern ourselves with extensions and generalizations of certain theorems on reduced range extensors and of their contractions.

The symbolism is essentially the same as used in the previous section. Here we shall denote two coordinate systems x^a and x^r respectively. Letters at the beginning of the alphabet ($a, b, c, d, e, \alpha, \beta, \gamma, \delta, \varepsilon$) will be associated with x^a coordinate system, while the remaining others including $i, j, k,$ $\lambda, \mu, \nu,$ with x^r system.

The existence of extensors of reduced range is illustrated by the following theorem

If $T_{c\ \delta d}^{\alpha a\ \beta b}$ is an extensor and $\theta \leqslant \alpha \leqslant M$, $\theta \leqslant \delta \leqslant M$, $\phi \leqslant \beta \leqslant M$ then the transformation equation of the quantities

$$\sum_{=\theta}^{M} \sum_{\beta=\phi}^{M} \binom{\alpha}{\theta} \binom{\beta}{\phi} T_{c\ \delta d}^{\alpha-\theta a\ \beta-\phi b}$$

may be put in the form

$$\sum_{\alpha,\rho=\theta}^{M} \sum_{\beta\,\sigma=\phi}^{M} \binom{\alpha}{\theta} \binom{\beta}{\phi} T_{c\ \delta d}^{\alpha-\theta a\ \beta-\phi b} X_{(\alpha)a}^{(\rho)r} X_{(\beta)b}^{(\sigma)s} X_{l}^{c} X_{(\lambda)l}^{(\delta)d}$$

$$= \sum_{\rho=\theta}^{M} \sum_{\sigma=\phi}^{M} \binom{\rho}{\theta} \binom{\sigma}{\phi} T_{l\ \lambda l}^{\rho-\theta r\ \sigma-\phi s}$$

Proof. By virtue of the relation [8]

$$X_{(\ )a}^{(\rho)r} = \binom{P}{A} X_{a}^{r\ (P-A)} \qquad P=\rho \quad A=\alpha$$

we see that $\binom{A}{\theta} X_{(A)a}^{(\rho)r}$ reduces to $\binom{P}{\theta} X_{(A-\theta)a}^{(P-\theta)r}$ and $\binom{B}{\phi} X_{(B)b}^{(\Sigma)s}$ reduces to $\binom{\Sigma}{\phi} X_{(B-\phi)b}^{(\Sigma-\phi)s}$

Therefore, by means of these relationships, the left member of the relation to be established becomes :

$$\sum_{\rho=\theta}^{M} \sum_{\sigma=\phi}^{M} \binom{\rho}{\theta} \binom{\sigma}{\phi} \Gamma_{l\ \lambda l}^{\rho-\theta r\ \sigma-\phi s}$$

after replacing α by $\alpha+\theta$ and β by $\beta+\phi$ and thus the theorem is proved.

If we delete the indices β b ϕ and therefore σs, ϕ in the above theorem, we have the following

If $T^{aa}_{c\ \delta d}$ is an extensor and $\theta \leq a \leq M$ then the transformation equation of the quantities $\sum_{a=\theta}^{M} \binom{\rho}{\theta} T^{a-\theta a}_{c\ \delta d}$ may be expressed in the form

$$\sum_{a=\theta}^{M} \binom{a}{\theta} T^{a-\theta a}_{c\ \delta d} \; X^{(\rho)r}_{(a)a} \; X^{c}_{t} \; X^{(\delta)d}_{(\lambda)l} = \sum_{\rho=\theta}^{M} \binom{\rho}{\theta} T^{\rho-\theta r}_{t\ \lambda l}$$

If we consider T^{aa} under this theorem we get a result obtained by Craig[18]

On making use of mathematical induction, we can easily generalise the above theorem.

The existence of extensors of reduced range gives idea of the contraction of extensors tensors and finally invariants over a reduced range. The general process has been illustrated in full generality by the following theorem:

If $T^{aa\ \beta-\theta b\ \gamma-\phi c}_{d\ \delta e\ \epsilon k}$ is an extensor of reduced range θ ... M $\theta \leq \delta \leq M$ $\phi \leq \gamma \leq M$ $\psi \leq \theta \leq M$ then the quantities $\sum_{\beta=\theta}^{M} \sum_{\gamma=\phi}^{M} \binom{\beta}{\theta} \binom{\gamma}{\phi}$ $T^{aa.\ \beta-\theta b\ \gamma-\phi c}_{d.\ \beta b\ \gamma c}$ are the components of a mixed extensor

Proof From the law of extensor transformation we have

$$(12.1)\quad \sum_{\beta=\theta}^{M} \sum_{\gamma=\phi}^{M} \binom{\beta}{\theta} \binom{\gamma}{\phi} T^{aa\ \beta-\theta b\ \gamma-\phi c}_{d\ \beta b\ \gamma c} = \sum_{\beta,\gamma,\lambda=\theta}^{M} \sum \binom{\beta}{\theta} \binom{\gamma}{\phi} T^{\rho r\ \sigma-\theta s\ \eta-\phi t}_{i\ \lambda j\ \xi l} \; X^{(a)a}_{(\rho)r} \; X^{(\beta-\theta)b}_{(\sigma-\theta)s} \; X^{(\gamma-\phi)c}_{(\eta-\phi)t} \; X^{i}_{d} \; X^{(\lambda)j}_{(\beta)b} \; X^{(\xi)l}_{(\gamma)c}$$

in which the part of the member of right hand side with dummy β i.e. $\sum_{\beta=\theta}^{M} \binom{\beta}{\theta} X^{(\beta-\theta)b}_{(\sigma-\theta)s} X^{(\lambda)j}_{(\beta)b}$ by means of the relation

$$\binom{\beta}{\theta} \binom{\beta-\theta}{\Sigma-\theta} \binom{\beta}{\Sigma}^{-1} = \binom{\Sigma}{\theta} \text{ and } X^{(\beta)b}_{(\sigma)s} X^{(\lambda)j}_{(\beta)b} = \delta^{\lambda}_{\sigma} \delta^{j}_{s}$$ reduces to

$\sum_{\sigma\lambda=\theta}^{M} \binom{\sigma}{\theta} \delta^{\lambda}_{\sigma} \delta^{j}_{s}$ and similarly the member with dummy γ reduces to

$\sum_{\eta\xi=\phi}^{M} \binom{\eta}{\phi} \delta^{\xi}_{\eta} \delta^{l}_{t}$ and hence (12.1) becomes

$$\sum_{\beta=\theta}^{M}\sum_{\gamma=\phi}^{M}\binom{\beta}{\theta}\binom{\gamma}{\phi}\Gamma^{\alpha a,\ \beta-\theta b\ \gamma-\phi c}_{d,\ \beta b\ \gamma c} = \sum_{\sigma=\theta}^{M}\sum_{\eta=\phi}^{M}\binom{\sigma}{\theta}\binom{\eta}{\phi} T^{\rho r\ \sigma-\theta s\ \eta-\phi t}_{t\ \sigma s\ \eta t}\, X^{(\alpha)a}_{(\rho)r}\, X^{t}_{d}$$

Thus the theorem is proved.

If the indices $\gamma-\phi c$ γc and therefore $\delta-\phi t$ δt are absent we get the result given by Craig[8]

If further αa d and therefore ρr t are absent, we get an example of invariance in functional form with respect to T There are, evidently $(M+1)$ contractions with regard to a pair of superscript and subscript. If $\theta=M$, we get the contraction of tensor analysis. If $\theta=O$ we get an analogous contraction. Moreover when $O \angle \theta \angle M$, we have contractions involving binomial coefficients Kawaguchi[4] has shown that there do not exist other kind of contractions.

The contraction over full and reduced ranges by means of quotient law is illustrated by the following theorem.

If for every differentiable vector V the set of quantities $\sum_{\gamma=\theta}^{M}\sum_{\delta=\phi}^{M}\binom{\gamma}{\theta}\binom{\delta}{\phi} T^{\alpha a,\ \beta b}_{c\ \gamma d\ \delta} V^{\gamma-\theta d\ \delta-\phi e}$ are components of a mixed extensor of the type indicated by the free indices then $T^{\alpha a,\ \beta b}_{c\ \gamma d\ \delta e}$ is a mixed extensor of reduced range $\theta \leqslant \gamma \leqslant M$, $\phi \leqslant \delta \leqslant M$.

Proof By virtue of the hypothesis, we have

$$\sum_{\gamma=\theta}^{M}\sum_{\delta=\phi}^{M}\binom{\gamma}{\theta}\binom{\delta}{\phi} T^{\alpha a,\ \beta b}_{c\ \gamma d\ \delta e} V^{\gamma-\theta d\ \delta-\phi e}$$

$$= \sum_{\lambda=\theta}^{M}\sum_{\mu=\phi}^{M}\begin{Bmatrix}\lambda\\ \theta\end{Bmatrix}\begin{Bmatrix}\mu\\ \phi\end{Bmatrix} T^{\rho r\ \sigma s}_{t\ \lambda l\ \mu k} V^{\lambda-\theta l\ \mu-\phi k}\, X^{(\ )a}_{(\rho)r}\, X^{(\beta)b}_{(\sigma)s}\, X^{t}_{c}$$

which, after replacing $V^{\lambda-\theta l\ \mu-\phi k}$ by

$$\sum_{\gamma=\theta}^{M}\sum_{\delta=\phi}^{M} V^{\gamma-\theta d\ \delta-\phi e}\, X^{(\lambda-\theta)l}_{(\gamma-\theta)d}\, X^{(\mu-\phi)k}_{(\delta-\phi)l}$$

and making use of

$$\sum_{\lambda=\theta}^{M}\binom{\lambda}{\theta} X^{(\lambda-\theta)l}_{(\gamma-\theta)d} = \sum_{\gamma=\theta}^{M}\binom{\gamma}{\theta} X^{(\lambda)l}_{(\gamma)d}$$

and

$$\sum_{\mu=\phi}^{M}\begin{Bmatrix}\mu\\ \phi\end{Bmatrix} X^{(\mu-\phi)k}_{(\delta-\phi)e} = \sum_{\delta=\phi}^{M}\begin{Bmatrix}\delta\\ \phi\end{Bmatrix} X^{(\mu)k}_{(\delta)}$$

reduces to

$$\sum_{\gamma=\theta}^{M}\sum_{\delta=\phi}^{M} V^{\gamma-\theta d\ \delta-\phi e}\binom{\gamma}{\theta}\binom{\delta}{\phi}\left[T^{a\alpha\ \beta b}_{c\ \gamma d\ \delta e}-T^{\beta r\ \alpha s}_{t\ \lambda l\ \mu k}\ \ldots\right]$$

which shows that the bracket must vanish under the conditions a[illegible] the theorem follows.

In an analogous way we can prove the following theorem

If for every arbitrary extensor $V_{a\alpha,\ \beta b}$ the set of quantities

$$\sum_{\alpha=\theta}^{M}\sum_{\beta=\phi}^{M}\begin{Bmatrix}\alpha\\ \theta\end{Bmatrix}\begin{Bmatrix}\beta\\ \phi\end{Bmatrix}T^{\alpha-\theta a\ \beta-\phi b}_{c\ \gamma d}\ V_{a\alpha\ \beta b}$$ are components [illegible]

extensor of the type indicated by free indices, then $T^{a\alpha,\ \beta b}_{c\ \gamma d}$ is a mixed ex[illegible]

of reduced rang $\theta\leqslant\alpha\leqslant M$ $\phi\leqslant\beta\leqslant M$ and

If the set of quantities $$\sum_{\alpha=\theta}^{M}\sum_{\beta=\phi}^{M}\sum_{\gamma=\phi}^{M}\sum_{\delta=\eta}^{M}\binom{\alpha}{\theta}\binom{\beta}{\phi}\binom{\gamma}{\phi}\binom{\delta}{\eta}\times T^{\alpha-\theta a\ \beta-\phi b}_{c\ \gamma d\ \delta e}\ V^{\gamma-\phi d\ \delta-\eta e}_{a\alpha\ \beta b}$$

are components of an extensor for arbitrary extensor

$V^{\gamma-\phi d\ \delta-\eta}_{a\alpha\ \beta b}$ then $T^{a\alpha\ \beta b}_{c\ \gamma d\ \delta e}$ is an extensor of reduced range $\theta\leqslant$ [illegible] M
$\phi \leqslant \beta \leqslant M$ $\phi \leqslant \gamma\leqslant M$ $\eta\leqslant\delta\leqslant M$

The last three theorems can b[e] generalized to higher order [illegible] to get the extensors and tensors of any order and finally [illegible] contractions over reduced and full ranges.

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MORPHOLOGICAL AND CROSS-INCOMPATIBILITY STUDIES IN SOME SPECIES OF *PSIDIUM**

J N SETH†
Govt. Horticultural Research Institute Saharanpur

Psidium a genus of family Myrtaceae sub-family Myrtoideae is native to tropical America The species *P guajava* commonly known as guava, is widely cultivated in India and covers a total of about 1 25 327 acres Its fruits are a very rich source of vitamin C and contain 300 to 450 mgs of ascorbic acid per 100 gm. of pulp The review of literature on *Psidium* shows that very little work has been done on the floral biology cytogenetical and morphological aspects, which are essential prerequisites to any successful hybridization for their improvement. The present work has been done with these objectives in view and deals with the external morphology floral b ology incompatibility cytology embryology and development of seeds of five species, viz., *P guajava*, *P guineense*, *P chinense*, *P molle* and *P cattleianum* var *lucidum*.

It is observed that these species show only slight differences in their floral biology There are only two blooms (1) summer bloom, and (2) rainy season bloom. *P guajava*, *P guineense* and *P molle* have also slight tendency to flower during January February The floral buds mature in about 28 to 35 days and set fruits in about 103.2 to 197.2 days Floral buds as well as fruits of *P chinense* mature in the longest period and those of *P cattleianum* var *lucidum* in the shortest time. Floral buds pass through six distinct stages of development.

Anthesis and dehiscence of anthers start from early morning (5 30 a. m.) The former is influenced by minimum temperature, while the latter by relative humidity In summer bloom most of the flowers wither in the bud stage and this has been found to be directly correlated with the maximum temperature of the day Observations on anthesis and dehiscence of anthers under controlled conditions also show that cent per cent flowers wither at 40° C and dehiscence of anthers is delayed by five to six hours at 100 per cent relative humidity than that of 25 per cent. In *P cattleianum* var *lucidum* dehiscence of anthers takes place after anthesis, whereas in other species generally it precedes anthesis.

Pollen viability of these species ranges from 54 4 to 98.2 per cent in aceto-carmine and 42.6 to 79·2 per cent in vitro Pollen grains of *P guineense* have the maximum viability whereas those of *P cattleianum* var *lucidum* possess the least. Germination of pollen grains has been tried in different concentrations (5 to 90%) of sucrose lactose and dextrose. Sucrose medium

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† Present address : Govt. Agricultural Research Farm, Mauranipur Jhansi.

has proved to be the best. The pollen grains of *P. molle* show the highest percentage of germination as well as pollen tube growth in 15 per cent sucrose, while those of remaining four species in 10 per cent. In lactose large number of pollen tubes burst just after growth whereas in dextrose cytoplasm oozes out from the pollen grains without formation of any pollen tubes. The pollen grains of *P. guajava*, *P. guineense* and *P. chinense* also germinate in tap water.

Germination of pollen grains as well as their tube length increases remarkably by adding 0.5 per cent agar to the sucrose solution. Addition of gibberellic acid, naphthalene acetic acid and indolebutyric acid also accelerates the pollen germination and tube growth, but the effect of the latter is more marked. The effect of these plant regulators however varies with different species. Addition of 20 to 30 ppm boric acid accelerates the pollen tube growth three to four times. Pollen tubes in plant regulators and boric acid show some morphological abnormalities like swelling, coiling and branching at the tip but inspite of this they continue to grow and generally do not burst. The optimum temperature for pollen germination is found to be 25°C for *P. cattleianum* var. *lucidum* and 30°C in other species.

The pollen grains of *Psidium* species remain viable for a day only under field condition and eight to twelve days at the room temperature. If stored in 0 to 25 per cent relative humidity at the room temperature they remain viable for twelve to twenty four days whereas at low temperatures (0 to 4.5°C) they exhibit longevity of 90 to 135 days. It is observed that the pollen grains of *P. cattleianum* var. *lucidum* show optimum longevity in 0 per cent relative humidity at 4.5°C, whereas those of *P. chinense* in 0 per cent relative humidity at 0°C. The pollen grains of remaining three species show the highest longevity under 25 per cent relative humidity at 0°C. Pollen longevity is the highest in *P. guineense* and the lowest in *P. cattleianum* var. *lucidum*. The stored pollen grains do not show any correlation between pollen germination in culture and fertilization tests.

The stigma of *P. cattleianum* var. *lucidum* becomes receptive from the day of opening and continues to be so up to 72 hours. In other species, the stigma becomes receptive one day before opening and remains so up to ... after anthesis. Fertilization takes place within four days of pollination. Fruit setting is very high during rainy season bloom in comparison to that of summer bloom.

P. molle plants studied by the author show gametophytic type of self incompatibility. In interspecific crosses some of the species show ... unilateral incompatibility whereas others exhibit reciprocal ... It is also observed that the polyploid species i.e. *P. cattleianum* var. ... (octaploid) and *P. molle* (tetraploid) do not cross with diploid species ... used as male parent. Reciprocal crosses are however compatible ...

incompatibility is of two types. In one type the cross fails due to the inhibition of pollen tube growth in the style as observed in self incompatible *P. molle*. In another type, the pollen tubes grow through the style and syngamy as well as triple fusion takes place normally. In several cases the endosperm becomes eight to ten nucleate after which the flowers wither. In the cross *P. guajava* x *P. cattleianum* var. *lucidum* always seedless fruits are formed.

Self-incompatibility from *P. molle* and cross-incompatibility from the crosses *P. molle* x *P. chinense* and *P. cattleianum* var. *lucidum* x *P. chinense* have been overcome by the sprays of 100 ppm gibberellic acid. A large number of fruits obtained in these treatments are however seedless, but a very small percentage of fruits with viable seeds are also met with. Fruits in these treatments become quite abnormal in shape and calyx at the apex also becomes fleshy which persists till ripening. Addition of 100 ppm boric acid to gibberellic acid further improves the setting in these crosses and also induce fruit and seed-setting in more incompatible crosses *e. g. P. guineense* x *P. chinense* and *P. guineense* x *P. cattleianum* var. *lucidum*. Application of 1 per cent indolebutyric acid in lanolin paste to the ovaries at the time of pollination, or slightly before it, also induced fruit and seed-setting in the incompatible crosses viz. *P. guajava* x *P. molle* and *P. molle* x *P. chinense*. In the latter cross only parthenocarpic fruits develop. It is concluded that these two plant regulators delay the flower shedding and thus allow enough time for the incompatible pollen tubes to grow through the style. Boric acid, on the other hand, helps by accelerating the rate of pollen tube growth.

Trimming the stylar end of ovaries up to the ovules and putting pollen grains along with sugar agar culture media on the cut surface is also tried. In all these treatments few seedless fruits are obtained in the self pollinated *P. molle* and cross *P. molle* x. *P. chinense*. In some of the fruits degenerated ovules form a hard structure in the centre appearing like the seed. It is also observed that fruits develop only in the pollinated ovaries and therefore it is concluded that pollination is very essential for the development of seedless fruits in *Psidium* species.

Cytology of these species has shown that *P. guajava*, *P. guineense* and *P. chinense* are diploid ($2n=22$) whereas *P. molle* is a tetraploid and *P. cattleianum* var. *lucidum* an octaploid.

Anther wall is made of five cell layers of which innermost layer is tapetum whose cells become binucleate at the time of meiotic mitosis in the microspore mother cells. The tapetum degenerates by the time young microspores separate from each other. In several cases the tapetal cells do not degenerate even when the young microspores acquire exine. In *P. chinense* often, the tapetal cells enlarge in size and project in the anther locule. In *P. molle* and *P. cattleianum* var. *lucidum* sometimes instead of tetrads of microspore more than four cells are formed.

Ovary is unilocular and generally three and in *P. ...* even up to six rows of ovules are borned on the flanks of parietal placentae. By the time floral bud matures these placentae fuse at the tips and ... The ovary therefore, appears tetra or penta-locular

Ovule is anatropous bitegmic and crassinucellate In *P. ...* and *P. guineense* casually ovules reach up to campylotropous stage only. Micropyle takes a zig zag course and is often also formed of only one integument. Ovule receives a single vascular supply which terminates at chalaza. Two ... embedded in a common set of outer and inner integuments are also observed in few ovules of *P. guineense*, *P. molle* and *P. guajava*.

The megaspore mother cell is discernible in the third or fourth layer of nucellus In some ovules of *P. molle*, *P. guineense* and *P. cattleianum* var. *...* two megaspore mother cells are formed. Occasionally both of these develop and form twin embryo sacs In normal ones a linear tetrad of megaspores is formed. The chalazal one of these is functional and forms eight nucleate embryo sac. Development of the embryo is of Polygonum type. Embryo sacs with varied arrangements of their nuclei are also common.

Endosperm is free nuclear and in the beginning has an aggregation of denser cytoplasm and larger number of nuclei at the chalazal end. Cell wall formation starts from the micropylar end and it takes place when zygote is dividing or it has formed a proembryo of globular stage Entire endosperm including its chalazal end becomes cellular but the whole of it is consumed by the developing embryo Embryogeny is highly abnormal in most of the zygotes. The development of embryo is of Onagrad type and mature embryo is somewhat horse shoe shape In *P. cattleianum* var. *lucidum* often the cells at the micropylar region become rich in cytoplasmic contents and proliferate simulating nucellar embryos which project inside the embryo sac. However mature embryo from this nucellar growth has never been ...

In post fertilization stages, ovule becomes ana amphitropous in shape. This is brought about by the projection of basal body in the arch of ... The micropyle therefore comes close to the chalaza. Two hypostases ... entiate at two different places in the nucellus. The first is ... the embryo sac and arises soon after fertilization while the second ... at the place of fusion of nucellus with the inner integument. The ... first hypostase are slightly thick-walled and poor in cyt... whereas those of second hypostase are filled with tannin and ... with safranin Only the second hypostase persists ... inner integument degenerates completely except ... accumulates in the micropyle

A mature seed coat is formed of two layers The ... hard is fifteen to twenty cell layered and is derived from the ...

outer integument, whereas the inner soft part, is formed of the inner epidermis of the outer integument and is two- to three layered. The cells of mesophyll degenerate early. Cells of chalaza, raphe and basal body help in the formation of hard part of seed coat. Nucellus is represented by its epidermis and one or two cell layers more. The swollen tips of outer integument get detached and in the micropyle form a cap protecting the embryo.

The gametogenesis of the species closely resemble to that of the members of Lythraceae, Onagraceae, Sonneratiaceae and Melastomaceae. It is thus concluded that on embryological grounds Myrtaceae appears to be closely related to these families.

ON THE SPIRUROID GENUS *PHYSALOPTERA* RUDOLPHI 1819 IN SOME OF THE INDIAN CARNIVORES—A PRELIMINARY NOTE

V P GUPTA AND B P PANDE
Department of Parasitology U P College of Vet. Sc & An Husbandry Mathura.

A very large number of species of *Physaloptera* are known as parasites of a great variety of vertebrates amongst which some are associated with gastric affection not only in domestic carnivores but also in wild ones including those in captivity Of these, *P brevispiculum* v Linstow 1906 *P fuelleborni* (Mirza, 1934) *P praeputialis* v Linstow 1889 (Syn. *P masoodi* Mirza 1934) and *P rara* Hall and Wigdor 1918 have been recorded from domestic carnivores in this country by Baylis and Daubney (1922 23) Chandler (1925) Korke (1928) Mirza (1934) Mirza and Singh (1934) Bhalerao (1935) Acharya (1939) Mudaliar and Alwar (1947) Thapar (1956) and Rao (1958)

During the course of faunistic survey on the helminth parasites of cat honey badger (*Mellivora indica* Kerr) fox (*Vulpes bengalensis*) and dog one of us (V P G) collected a large number of physalopterids The specimens from cat, consisting of mostly adults and a few juveniles were found to belong to *P fuelleborni* which, on study showed that the account given by the author in certain respects needed a redescription. As the worms were found attached to the mucosa of the stomach, pieces of gastric wall were suitably fixed for subsequent histopathological study to assess the host-parasite relationship which too have been attempted in this paper From one of the three badgers, examined post mortem, the single specimen obtained was an adult female which resembled completely the female worms collected from cat. The specimens obtained from the stomach of fox have on examination proved to belong to a new species which has been named provisionally as *P vulpineus* and is described briefly The two specimens recovered from one of the seventy seven dogs autopsied, were immature and the one under ecdysis had around it the loose sheath of its previous stage. A specific determination, in the absence of adults is not possible and these juveniles are briefly described here as *Physaloptera* sp

1 *P fuelleborni*

The male specimens 20-35 long and 0.75-1 42 in maximum thickness have transverse cuticular striations throughout. The mouth is with two rounded lips, each having two papillae on its external surface and with a large external and an internal tooth of the same height as the external one but tridigitate in character The oesophagus 3 7 5 3 in length, has a smaller muscular

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part followed by a larger glandular posterior portion. The nerve ring lies at 0.42-0.59 the cervical papillae at 0 67 1.00 and the excretory pore at 0.73-1.15 distance respectively from the anterior extremity The caudal end with lateral alae and a tail of 0 87 2.17 length has its ventral surface from infront of the cloacal opening to near the posterior end covered with continuous longitudinal ridges In addition to the usual four pairs of pedunculated lateral papillae in the pre and post cloacal regions there are sixteen sessile papillae of which the three precloacal ones lie transversely on the anterior lip of the cloaca with the median papilla largest in this series and the four situated immediately behind the cloacal aperture, are arranged crescentically and of these the two median ones are relatively smaller than the laterals. The remaining nine of the sessile papillae, situated in the posterior half of the tail are arranged in four transverse rows the first two rows with two papillae in each constituting the anterior group which infront and around, has a characteristic longitudinal ribbed-area, separated by a distinct gap from the rest of the papillae which too are in two rows and lie close together the first row has the two papillae which are smaller in size than the papillae behind which are however three in number with the median one the largest. The spicules are very unequal in size, the left thinner and longer and measures 1 43-3 1 while the right one slightly stouter but shorter measures 0.5-1.29 in size

The female measures 28-42 in length and 0 97 1.38 in maximum width. The oesophagus is 5 7-6.9 in length. The nerve ring lies at 0.4 -0.6 the cervical papillae at 0 67 0 85 and excretory pore at 0 7?-0.95 distance respectively from the anterior extremity The vulva situated at 11.56-13.5 distance from anterior and is covered by a detachable ring of brown [illegible] and leads into thick muscular vagina which joins posteriorly the [illegible] thicker egg chamber Vagina including egg chamber measures [illegible] in length The two uteri arise posteriorly from the egg chamber [illegible] horns and run parallel. The eggs inside vagina are almost round [illegible] measure 33-37 x 29-33μ in size

Juvenile males—The specimens measure 13-14 in length with a maximum width of 0 65 Cuticle shows transverse striations. The two lips are [illegible] formed with the typical adult characters. The oesophagus measures [illegible] The nerve ring lies at 0.52-0 53 and the excretory pore at 0.55-0.58 [illegible] respectively from the anterior end The caudal extremity does [illegible] developed alae as in the adult but the usual number of papillae the [illegible] of the spicules and the testicular region have made their appearance [illegible] one of the specimens, appearing younger the sessile papillae [illegible] of spicules and testis alone were seen Tail is 0.37-0.55 in length.

Juvenile female—The specimen recovered measures ?1 with a [illegible] width of 0 75 The oesophagus is 3 180 long The nerve ring [illegible] cervical papillae at 0 69 and the excretory pore at 0 5 distance [illegible]

anterior end. The genital pore is situated 7.73 distance behind the anterior end. The muscular vagina, with fully formed egg chamber measures 1.95 in length and shows the two uteri arising as its two horns. The tail is 0.3 in length.

Histopathology—The worms firmly attached to the mucosa of stomach have a covering of mucus. Histological study revealed that the worms, for burrowing deep into the mucosa use their teeth and from the destruction of the gastric glands in this region a reduction of its effective surface results. Both the intact as well as damaged mucosa have copious mucus which is secreted on account of irritation caused by the worms which, in heavy infestations, may lead to grave gastric troubles. No marked cellular elements in and around the areas of attachment were however observed.

As quoted Baylis (1939) *P. fuelleborni* was originally described by Mirza as *Chlamydonema fuelleborni* and the genus *Chlamydonema* differentiated from *Physaloptera* on the presence of prepuce like sheath both in males and females, is a synonym of *Physaloptera*. In dealing with *P. brevispiculum* Baylis also stated that one of the four pairs of the papillae, described and figured by Mirza perhaps represented the "caudal pores" and accordingly believed that this species was a synonym of *P. brevispiculum*.

From a study of the large number of specimens available with us it is evident that the fourth pair described by Mirza is a feature constantly present and there is thus no justification to consider these as pores. The species *P. fuelleborni* therefore, stands valid. Some variations however have been observed in our material from the original description for this species and these relate to the oesophagus which has been described with a constriction in its posterior part in male specimen studied by Mirza. This feature was not encountered in any of the specimen available with us. The second point of difference is in regard to the papillae as amongst the sessile papillae, the two median papillae of the immediately post-cloacal series have been found, to be relatively smaller than the laterals. The first and third row of the tail papillae are also relatively smaller than the second and fourth series which have been described with only two papillae but really are three in number. Possibly these points were overlooked in the only specimen available to the author for examination.

A single adult female recovered from the honey badger on study was found to resemble in all respects the female worms collected from the cat. The species *P. fuelleborni* has therefore been found to occur in this host which is a first record of its occurrence in this animal.

P. tuplaceus n. sp.

Three males and one female specimen of a physalopterid species were collected from one of the three foxes examined for parasites. The specimens on study appeared distinct from the species so far known under the genus

Physaloptera and are described below as *P. vulpineus* n. sp. which is differentiated from the other known forms.

Males—The specimens measure 15-25 in length with a maximum breadth of 0.36-0.759. The cuticle is transversely striated. The two rounded lips each with two papilla on its external surface, have one external and three internal teeth of nearly the same size. The oesophagus, with its anterior shorter but muscular and a longer glandular posterior part, measures 4-4.9 in length. The nerve ring lies at 3.5-5.8, the cervical papillae at 0.4-0.50 and excretory pore at 0.50-0.77 distance respectively from anterior end. The caudal end with lateral alae and a tail of 0.7-1.4 length bears on its ventral surface beginning from infront of cloacal aperture to just behind the posterior extremity continuous longitudinal ridges formed by tubercles. In addition to the usual four pairs of pedunculated lateral papillae there are thirteen pre- and post-cloacal sessile papillae, three of which are precloacal and arranged in the form of 'V' with the median one the smallest in the group, lying just on the anterior lip of the cloaca while the two lateral ones are of equal size. Just behind the cloacal opening, the four sessile papillae lie arranged crescentically and are of nearly the same size. The three pairs of sessile papillae, lying behind the pedunculated series, are present on the posterior portion of the tail, being nearly equidistant from each other. The first two pairs of the series lie in the ribbed area while the third one is posterior to it. The two spicules, of similar shape and size measure 0.44-0.49 in length.

Female—The specimen measures 28.5 in length and 0.91 in maximum thickness. The oesophagus is 6.0 in length. The nerve ring is situated at 0.48, the cervical papillae at 0.68 and the excretory pore at 0.69 distance respectively from anterior end. The genital pore in the oesophageal region lies at 4.22 distance from anterior end. The muscular vagina is 3.09 long. The two uterine tubes, arising directly from egg chamber run posteriorly but parallel to each other. The bluntly ending tail is 0.49 in length. The fully formed eggs, inside the vagina, are 30-40 × 23-30 µ in size.

HOST—*Vulpes bengalensis* (fox)

HABITAT—Stomach

LOCALITY—Mathura

The type specimens deposited in the collection of the Department of Parasitology U. P. College of Veterinary Science & Animal Husbandry, Mathura, U.P. India.

Remarks—A very large number of species of *Physaloptera* known from reptilian, avian, and mammalian hosts have been described by various workers and amongst the numerous scattered references the description of new species or redescriptions of some of the earlier known forms by ... Ortlepp (1922, 1937), Hill (1940) and Morgan (1943) have ...

hensive details about the species considered valid by them To the first we owe a useful key to the sufficiently known forms. The present specimens are assignable to the group of species with two uteri which are included under *Didelphys* As the origin of two uteri is directly from the egg chamber and the position of vulva is in the oesophageal region towards the anterior part of the body the present material appears to resemble the species *P tacapensis* Seurat, 1917 *P immerpani* Ortlepp 1937 and *P getula* Seurat, 1917 On account of the spicules in the material from fox being equal and difference in the arrangement of the sessile papillae, the specimens appear quite distinct from these three forms and are accordingly assigned to a new species for which the name *P vulpineus* is proposed

The two immature specimens collected from one of the dogs belong to *Physaloptera*. The specimen, under ecdysis, measures 8 5 in length and has a maximum breadth of 0·528 The cuticle has transverse striations The two lips are fully formed. The oesophagus, in two muscular and glandular parts, measures 2 662 in length The nerve ring lies at 0.28 and cervical papillae at 0·53 distance respectively from the anterior extremity

The second specimen, in a more developed stage, measures 9·0 in length and has a maximum width of 0 58. The usual transverse striations are present. The lips are fully formed. The oesophagus measures 2 57 in length The nerve ring lies at 0·29 the cervical papillae at 0 533 and the excretory pore at 0·37 distance respectively from anterior end. The rudiments of male genitalia, though formed, do not seem to be fully demarcated

(All measurements except otherwise stated are in mm.)

(Figures 1—10 are camera lucida drawings)

ACKNOWLEDGMENT

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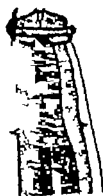


Fig 1

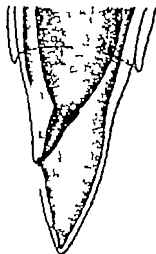


Fig 4

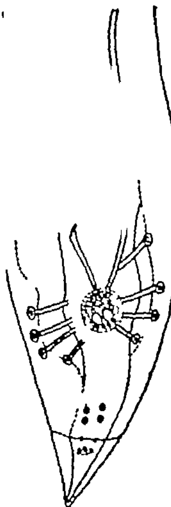


Fig 2

Fig 1 *Physaloptera fuelleborni* —anterior end.

Fig 2. P *fuelleborni*—caudal end of male.

Fig 4 P *fuelleborni*—caudal end of female.





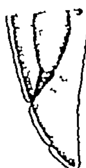




Fig 3 *P fuelleborni*—vulvar region of female.

Fig 5 *P vulpineus*—anterior end.

Fig 6 *P vulpineus*—caudal end of male

Fig 7 *P vulpineus*—caudal end of female.

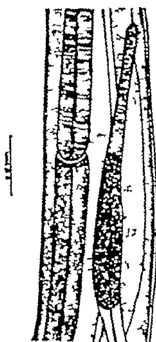

Fig 8


Fig 9

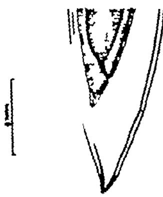

Fig 10


Fig 11

Fig 8. *P rulpecans*—vulvar region of female
Fig. 9. *P* sp of dog—anterior end of specimen under moult.
Fig 10 P sp —caudal end of (9)
Fig. 11 Photomicrograph—cross section of stomach of cat showing anterior end P fuelleborni deeply burried in the gastric mucosa 175 X

STUDIES ON MICROFLORA OF THE RESPIRATORY TRACT OF POULTRY WITH SPECIAL REFERENCE TO PLEURO PNEUMONIALIKE ORGANISMS*

SATISH CHANDRA ADLAKHA
Department of Pathology & Bacteriology
U P College of Veterinary Science & Animal Husbandry Mathura India.

The present study was undertaken to investigate the bacterial flora of the respiratory tract associated with chronic respiratory disease (CRD) of fowls and to establish the incidence of infections caused by pleuropneumonialike organisms (PPLO) in our country by cultural and serological means to find out the pathogenicity of these organisms and to suggest ways and means to control the disease (CRD complex) caused by them.

The bacterial flora of the respiratory tract of 150 birds from a flock which had a history of CRD comprised mostly of Gram negative organisms and PPLO Coliform organisms predominated the Gram negative isolates and formed about 51% of the total. Gram negative organisms seemed to play the role of secondary invaders in air-sac infection. No significance was attached to the presence of gram positive organisms.

Six poultry farms located at Mathura, Delhi, Chak-Ganjaria (Lucknow) Bharari (Jhansi) Babugarh (Meerut) and Ingraham Institute (Ghaziabad) were visited during the course of this investigation The flocks at all of these farms except the one maintained near Ghaziabad were all heavily infected, more than 50% of the birds were reactors to the PPLO diagnostic serological test as found by the 10% random sample survey Surprisingly the Ghaziabad farm was completely free of infection which was an encouraging observation. A few serum samples received from one of the poultry farms in Andhra Pradesh were found to be positive. PPLO infection seems to be widespread in the country

A fairly high degree of correlation was observed between the results of cultural isolation of PPLO and serology which indicated the utility of the rapid serological test fo detecting overt or inapparent avian PPLO infections Since infection with PPLO does not necessarily result in any clinical symptoms serological testing was found to be useful to determine if a flock was PPLO-free.

An interesting information was revealed when the ducks maintained with the fowls at some of the farms were tested for PPLO infections. All were found to be culturally and serologically negative. The possibility was suggested

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that either the ducks were resistant to such infections or they harboured strains antigenically different from the fowl strains

Broadly speaking, the biochemical and serological tests [illegible] the probable presence of potential pathogenic strains. Serological studies [illegible] 2 types of PPLO strains, Haemagglutination (HA) + and HA – [illegible] HA + strains most probably represented potential pathogenic strains, the HA [illegible] strains could not be considered innocuous since the only safe flock was a PPLO-free flock

The results of the pathogenicity trials suggested that uncomplicated PPLO infection in chickens remained inapparent was a relatively mild disease and was accompanied by singificant positive serological reactions for PPLO. In combination with other known pathogens such as *E coli* they played an im[illegible]tant role in the pathogenesis of such diseases as CRD and air-sac infection. Since the present experimental studies were conducted on a limited number of culturally and serologically negative chicks the necessity of carrying out further studies on chicks on a large scale, from a PPLO-free flock is indicated.

The results of isolation of PPLO from embryonated eggs, young chicks and breeding hens suggest that some degree of transovarian transmission of PPLO does occur However the comparative freedom of chicks from PPLO infection as compared to the adult breeding stock is suggestive of a method of breaking the cycle of infection.

THE PRINCIPAL MAGNETIC SUSCEPTIBILITIES OF RARE EARTH IONS IN CRYSTALS AT LOW TEMPERATURES*

D Neogy†

Physics Department Agra College Agra.

Though the magnetic studies on the rare earth ions in crystals are limited, they go fairly well to show a significant departure from the free ion values and the uniqueness of their magnetic behaviour in definite contrast to those of the salts of the iron group of elements. In support of the first statement we may quote the large observed anisotropies of the crystals which clearl indicate that the ions are no longer free and in justification of our second remark it may be pointed out that in the case of the rare earth ions the effective magnetic moment is more or less near its free ion value showing the weak influence of the crystal field on the ions whereas the field acting on the ions in the salts of the iron group are so strong as to quench the orbital part of the moment almost completely leaving only the spin free

Unfortunately no concerted effort has been made towards solving these problems. While the ethyl sulphates have been studied quite exhaustively by paramagnetic resonance methods not much work has been done to study their susceptibilities on the other hand the hydrated sulphates for which considerable amount of magnetic data are present little is known about their paramagnetic resonance phenomena. The theory of the ethyl sulphates is in quite advanced stage but no adequate theory has yet been worked out for the sulphates, mainly for the want of paramagnetic resonance data for these salts. Hence, in this thesis an attempt has been made to develop a theory in the same line as has been adopted for the ethyl sulphates (Elliot and Stevens 1953 Proc. Roy Soc. 219 387) but using the susceptibility values instead of PMR data.

The salient features of the theory with special reference to the main differences from iron group ions are as follows

(1) The magnetic carriers are 4f and not 3d electrons.

(2) The symmetry of the field is entirely different and certainly not even nearly cubic. This was pointed out as long back as 1939 (Penney and Kynch Proc. Roy Soc. 1939 170 112) when the cubic symmetry was found to be quite inadequate to explain the behaviour of sulphates. There is no X-ray data showing the crystal structure of these salts but from different considerations they are expected to possess similar paramagnetic units as those present in the Tutton salts. The ions seem to have six nearest neighbours arranged

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† Present address—Physics Department Burdwan University Burdwan, (W B.)

in the form of an octahedron having a vertical four fold axis of symmetry with a possible horizontal plane of reflection

(3) The crystal field splittings are much smaller in magnitude (100 cm^{-1}) than those in the iron group and in most cases lower than those produced by the spin orbit coupling This does not imply that the fields in rare earth salts are weaker but the effect of the field on the ion is diminished by the fact that the 4f electrons lie deep inside the ion, shielded by the closed shells of 5s and 5p electrons and partly because r^n values are smaller than those in the iron group The field is thus taken as a perturbation on degenerate terms which are eigenstates of J the total angular momentum unlike in a case where L and S are taken to be quantized separately in the crystal field consistent field and consequently neglects the exchange effects between different atoms. This approximation is fully warranted in the case for the magnetically diluted salts of the rare earth group such as those studied in this thesis

The matrix elements of the crystal field are obtained by the equivalent process of Stevens (Proc Phys Soc. 1952, 65 209) and not by direct use of the Wigner co efficients which is rather cumbersome Special ... methods had to be employed for solving the energy value problems of ... ions The susceptibilities are then calculated by using the expression given by Van Vleck (1932 The theory of electric and magnetic susceptibilities Oxford pub.)

The outstanding advantage that the magnetic studies on single crystals have over those on powders and in the state of solution lies in the fact that these give direct information as to the nature of the field ... about the paramagnetic ion It is true that all the paramagnetic ... not parallel and there will be some averaging of the contributions of different units towards the crystal anisotropy yet much information ... from this resultant anisotropy Furthermore, a knowledge of the ... anisotropies obtained by comparing the theoretically deduced ... paramagnetic units with those observed for the crystals allows us to draw ... useful conclusions regarding the orientations of the paramagnetic ... the unit cell of the crystal

If one loses sight of the anisotropic behaviour of the crystal ... guided simply by the mean susceptibilities ... apt to be ... developing a theory for the same as it is seen that crystal fields ... differing in nature and strength may account for the ... the mean susceptibility whereas the anisotropies are ... sensitive to the symmetry of the field and their strength ... field with definite characteristics can explain the observed ... in determining the field uniquely

Penney and Schlapp (1932 Phys Rev 41 194) and Miss Frank (Phys. Rev 193 48 771) fell victim to the same fallacy in trying to develop a theory for the rare earth ions in sulphates. They obtained a good fit with the observed powder values which gave only the mean susceptibility on the assumption of a cubic field. Existence of a cubic field essentially assumes isotropic character of the magnetic properties of the crystal which the measurements on the single crystals prove certainly not to be so

Therefore, while developing the theory in the present thesis main emphasis has been laid on the behaviour of the anisotropies of the crystals rather than on the nature of variation of the mean magnetic moments. However it may be seen that a theory which provides a good explanation for the anisotropies also accounts fairly well for the mean moments.

The single crystals of the octahydrated sulphates of the rare earth ions Ytterbium, Erbium, Dysprosium and Europium having a general formula $M_2(SO_4)_3.8H_2O$ have been studied for their principal magnetic susceptibilities at different temperatures from room temperature down to about 100°K i e within the range of liquid air temperature.

The anisotropies have been measured by the Krishnan and Banerji flip angle method. The absolute susceptibilities are determined by an electrodynamic microblance developed by the author using constant field gradient pole pieces of Dutta Roy The facilities for the low temperature work were available to the author in the Magnetism Department Laboratories of I.A.C.S. (Calcutta) through the kindness of Professor A. Bose of the same department The substances used for preparing the crystals were of spectroscopic pure variety especially imported from Johnson and Matthey Ltd. London.

The results obtained both experimentally and theoretically allow the following conclusions to be drawn :

(1) The observed anisotropies of all the four rare earth ions increase rapidly with the fall of temperature. In the case of Er^{+++} ion it is maximum the value at 100°K is nearly nine times the value at 300°K and hence seems to follow $\left(\frac{1}{T}\right)$ law This shows that the principal magnetic susceptibilities in different directions increases at different rates. In almost all cases the increase of the mean susceptibility is slightly less than what should be expected of a free ion. Thus a small but progressive fall of the effective magnetic moments is observed for Yb^{+++} Er^{+++} Dy^{+++} with lowering of temperature.

(2) In the case of Eu^{+++} ion the increase of the mean susceptibility with decrease of temperature is very small partially due to the depopulation of the higher J levels which contribute towards the paramagnetism of the

ion but mainly because of the large contribution towards the [illegible] of the second order Zeeman term (κ_1) for the ground state (J=0 [illegible]) independent of temperature. Consequently a sharp fall in the effective magnetic moment of the ion is observed which is not very much [illegible] from the expected free ion behaviour showing once more the ineffectiveness of the crystalline electric field on the mean moment of the ion.

(3) Attributing all the observed deviations in the magnetic behaviour of the ion in crystals from those of the ideal free ion state to the splitting of the ground state under the influence of the crystalline electric field possessing a tetragonal symmetry it is seen that the results of the magnetic measurements carried out on the ions Yb^{+++}, Er^{+++}, Dy^{+++} and Eu^{+++} are explained quite satisfactorily. A theoretical quantitative estimation for the first three ions show a very good agreement with the observed behaviour of the anisotropies. Also the variation of the mean magnetic moment with temperature as calculated theoretically follow close the experimental values.

(4) While trying to derive the observed magnetic anisotropies of the crystals from the theoretical values, it was necessary to assume certain definite orientations of the paramagnetic units inside the unit cell of the crystal. This is very significant in the sense that even without probing into the crystal structure by direct means such as X-rays etc. we are able to get some idea as to the same, simply from the magnetic measurements.

(5) In general the values of the parameters C_n^m are found to decrease as we go from Dy^{+++} ion towards Yb^{+++} ion in the rare earth series but no [illegible] rule seems to hold for the same unlike the case with the ethyl sulphates (E[illegible] and Stevens loc. cit.) The magnitude of r decreases as we move down the table of the rare earth series and hence if the field strengths remain the same in all the isomorphous salts we should expect a gradual decrease in the C_n^m values as well, in moving from Cerium to Ytterbium. In the present investigation it is found that C_2^0 parameter which is quite large for the present series of salts does not seem to follow any such rule which hints to the fact that perhaps the second order field strength varies considerably from salt to salt.

(6) For Er^{+++} ion the calculated energy level separations [illegible] well with the observed values of Meehan and Nutting though [illegible] the same. The low values of the crystal field parameters which may [illegible] observed levels do not explain the magnetic results. However a [illegible] delicate rearrangement of the values of three parameters [illegible] desired agreement with both magnetic results and the absorption [illegible] absolutely ruled out but such a possibility seems remote. [illegible] assignment of the observed absorption level are to be corrected.

(7) In the crystals of dysprosium and ytterbium with the lowering of temperature an appreciable change in the direction of the magnetic axes with respect to the crystal axes have been observed. Though in the absence of any X ray data a correct explanation to the above phenomenon is not possible it definitelyindicates some change in the crystalline structure of the atom with change of temperature, the most probable change that may be expected to take place insid. the crystal, is the variation in the orientations of the paramagnetic units in the unit cell

BACTERIOLOGICAL STUDIES ON MILK FROM COWS AND BUFFALOES WITH SPECIAL REFERENCE TO VARIOUS SEROTYPES BELONGING TO GROUP B AND C STREPTOCOCCI*

S. N BAKSHI†

Department of Pathology & Bacteriology

U P College of Veterinary Science & Animal Husbandry Mathura

The study under report was conducted to find out the microflora of udder milk from cattle and buffaloes. The investigation was specially aimed at finding out the serotypic pattern of group-B and group-C streptococci and the antigenic relationship between the various species belonging to group-C streptococci.

During the course of the investigation 1102 quarter milk samples from 276 cows and buffaloes belonging to the State organised herds located at Mathura, Madhurikund, Babugarh, Chakganjeria and Kalsi in the Uttar Pradesh were examined and 712 cultures of microorganisms were isolated. Streptococci isolated from milk were also received from Research Officer Mastitis Investigation Scheme, U P., Lucknow The various organisms isolated were *Str agalactiae Str dysgalactiae Str Zooepidemicus Str equisimilis*, streptococcus group-E streptococcus group-G strepto coccus group-L, *Str Uberis Str bovis, Str M. G Str thermophilus Str mitis Str acidominimus Str viridans*, unclassified streptococci, *Staph. aureus Staph. albus Staph. citreus E. coli* Klebsiella aerobacter group intermediate forms of coliform *C pyogenes C bovis Ps. aeruginosa* yeast, anthracoids and chromobacter

Str equisimilis was found to be fairly well distributed in the various herds of the state and a significant percentage of its isolations were associated with a rise in leucocyte count of milk.

Streptococcus group-E is being reported for the first time in the country and this group does not seem to be widely distributed in the dairy herds.

The isolation of *Str mitis Str M G* and *Klebsiella*—aerobacter group from milk is being reported for the first time in the country

The non haemolytic diphtheroids generally isolated from milk were found to be biochemically undistinguishable from *C bovis*.

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* An abstract of the thesis submitted as partial fulfilment for the award of M. V Sc. degree in Bacteriology Agra University

† Present address—Research Officer Animal Husbandry Department, Uttar Pradesh Badshahbagh, Lucknow

The haemolytic characters of various streptococci have been studied. One strain of *Str agalactiae* was found to be gamma haemolytic and all the strains of *Str dysgalactiae* were alpha haemolytic. It is suggested that in the systematic investigation of mastitis the colonies morphologically similar to streptococcus but manifesting alpha or gamma haemolytic characters should not be ignored.

The role of various biochemical reactions in the classification of streptococcus is discussed

Camp activity was manifested by all the *Str agalactiae* strains except one. Some strains of streptococcus group-E, *Str uberis* and *Str bovis* also exhibited this activity The Camp activity of *Str bovis* is probably being reported for the first time.

Fibrinolytic activity was observed in some of the *Str equisimilis* strains under study It seems that fibrinolytic activity is a variable character in animal strains and irrespective of the source of isolation may be associated with the antigenic make up of the strains.

Rise in leucocyte count of milk did not show complete parallelism with isolation of pathogenic bacteria It is suggested that in natural cases rise in leucocyte count above 200 000 per c. c. accompanied with isolation of pathogenic bacteria may be considered to be indicative of establishment of infection after crisis.

The serotype classification of the isolated *Str agalactiae* revealed that serotypes Ia III X and R exist in this country The record of serotype Ia is interesting as it is generally associated with human infections and so far it has been reported only from United States from animals. Serotypes Ia, X and R are being reported for the first time in India.

Serological studies of the various group-C species indicated that all the strains gave precipitation reaction with Wellcome Research Laboratories group-C sera as well as locally prepared sera against *Str dysgalactiae*, *Str zooepidemicus* and *Str equisimilis* The precipitation reaction did not show any parallelism with slide agglutination reaction. Two strains of *Str dysgalactiae* and six strains of *Str equisimilis* did not show agglutination with *Str dysgalactiae* antiserum Five strains of *Str equisimilis* did not agglutinate with *Str zooepidemicus* antiserum It was observed that *Str dysgalactiae* antiserum gave weaker precipitation (in tube or gel) as well as agglutination reaction as compared with *Str zooepidemicus* or *Str equisimilis* antisera.

Studies on antigenic fractionation and serological relationship between *Str dysgalactiae* *Str zooepidemicus* and *Str equisimilis* were conducted by precipitation test in tube and double diffusion in agar gel with the sera cross absorbed with the heterologous organisms.

On the basis of reaction with cross absorbed sera *Str dysgalactiae* could be divided into 3 types and *Str equisimilis* into 5 types The only strains of *Str zooepidemicus* isolated was similar in reaction to the N C T C strain.

The antigen of *Str dysgalactiae* giving precipiting reaction with absorbed sera I II IV and VI were trypsin and pepsin labile.

The antigen of *Str zooepidemicus* giving reaction with absorbed sera III and V was trypsin resistant but pepsin labile and was similar to R antigen in nature.

The antigens of *Str equisimilis* giving reaction with sera I and III were trypsin and pepsin labile The delayed reaction of trypsinised extract of N.C.T C. strain of *Str equisimilis* with sera III was due to the antigen R. The antigen giving reaction with sera V and VI was trypsin pepsin and heat resistant and was polysaccharide in nature.

The results of precipitation in agar gel indicated that in addition to the group specific C-polysaccharide, *Str dysgalactiae St zooepidemicus* and *Str equisimilis* shared tryspin and pepsin labile antigens *Str zooepidemicus* and *Str equisimilis* shared a trypsin resistant pepsin labile antigen and *Str equisimilis* had a trypsin pepsin and heat resistant, species specific antigen resembling polysaccharide in nature. The type specific antigens of *Str dysgalactiae* and *Str equisimilis* were trypsin and pepsin labile.

Str dysgalactiae was found to be comparatively less pathogenic for mice than *Str zooepidemicus* or *Str equisimilis* *Str equisimilis* could be regularly isolated from the spleens of infected mice after fifteen days but *Str dysgalactiae* and *St zooepidemicus* could not be isolated.

Str zooepidemicus and *Str equisimilis* seemed to be poor portecting antigens.

This report about the antigenic relationship between the three species belonging to group-C streptococcous is probably the first of its kind. The presence of common protein antigens in *Str dysgalactiae Str zooepidemicus* and *St equisimilis* sharing of R antigen by *Str zooepidemicus* and *Str equisimilis* and the species specific polysaccharide of *Str equisimilis* is being reported for the first time.

The haemolytic characters of various streptococci have been studied. One strain of *Str agalactiae* was found to be gamma haemolytic and all the strains of *Str dysgalactiae* were alpha haemolytic. It is suggested that in the systematic investigation of mastitis the colonies morphologically similar to streptococcus but manifesting alpha or gamma haemolytic characters should not be ignored.

The role of various biochemical reactions in the classification of streptococcus is discussed.

Camp activity was manifested by all the *Str agalactiae* strains except one. Some strains of streptococcus group-E, *Str uberis* and *Str bovis* also exhibited this activity. The Camp activity of *Str bovis* is probably being reported for the first time.

Fibrinolytic activity was observed in some of the *Str equisimilis* strains under study. It seems that fibrinolytic activity is a variable character in animal strains and irrespective of the source of isolation may be associated with the antigenic make up of the strains.

Rise in leucocyte count of milk did not show complete parallelism with isolation of pathogenic bacteria. It is suggested that in natural cases rise in leucocyte count above 200 000 per c. c. accompanied with isolation of pathogenic bacteria may be considered to be indicative of establishment of infection after crisis.

The serotype classification of the isolated *Str agalactiae* revealed that serotypes Ia, III, X and R exist in this country. The record of serotype Ia is interesting as it is generally associated with human infections and so far it has been reported only from United States from animals. Serotypes Ia, X and R are being reported for the first time in India.

Serological studies of the various group-C species indicated that all the strains gave precipitation reaction with Wellcome Research Laboratories group-C sera as well as locally prepared sera against *Str dysgalactiae*, *Str zooepidemicus* and *Str equisimilis*. The precipitation reaction did not show any parallelism with slide agglutination reaction. Two strains of *Str dysgalactiae* and six strains of *Str equisimilis* did not show agglutination with *Str dysgalactiae* antiserum. Five strains of *Str equisimilis* did not agglutinate with *Str zooepidemicus* antiserum. It was observed that *Str dysgalactiae* antiserum gave weaker precipitation (in tube or gel.) as well as agglutination reaction as compared with *Str zooepidemicus* or *Str equisimilis* antisera.

Studies on antigenic fractionation and serological relationship between *Str dysgalactiae*, *Str zooepidemicus* and *Str equisimilis* were conducted by precipitation test in tube and double diffusion in agar gel with the sera cross absorbed with the heterologous organisms.

STUDIES IN PARACHOR*

Suresh Govind Khandekar
Holkar College Indore

Introduction

In 1923 Macleod discovered a *simple* empirical relationship between surface tension of a liquid ( ) and it s density (D) thus

$$C = \frac{r^{\frac{1}{4}}}{(D-d)}$$

Where d is the density of the vapour at the *same temp at which* 'r and 'D are measured. C is a constant, characteristic *of the particular* substance and is independent of temp over a considerable range.

Sugden (1924) revised the equation to

$$P = \frac{Mr^{\frac{1}{4}}}{(D-d)}$$

Where P is a constant, *called* parachor and M is the molecular weight In essence P becomes the molecular *volume* At most temps the density d of the vapour is negligibly small and *may be* neglected in comparison with 'D' Hence

$$P = \frac{Mr^{\frac{1}{4}}}{D}$$

Sugden further showed by other considerations that parachor is really equivalent to molecular volume and that it is an additive property He and his co-workers studied the parachor of a large number of chemical compounds (in particular those which were isomeric with similar constitution belonging to different homologous series and involving various types of structures) and established parachor *constants* for different groups, atoms, valency bonds and structures

I have in my thesis used the Sugden s equation and his parachor values of atomic and structural parachors

Experimental

The determination of parachor involves two essential parts of the experimental procedure (a) determination of density in liquid state and (b) determination of surface tension. I have used for my work Sprengle s pyknometer

*Summary of the thesis submitted to the Agra University Agra for the degree of Doctor of Philosophy

for measuring density for it combines accuracy with suitability whenever densities of pure liquids or solutions are required. The surface tension was determined by Jaegar's method, which is based on the measurement of pressure required to form air bubbles in a liquid by means of capillary immersed vertically in it. This method is advantageous because surface tension of any suitable liquid or solution can be measured at atmospheric pressure over a wide range of temperature. Besides this method is simple and accurate. I have, therefore used this method in my work.

The purity of substances used was tested before use. The apparatus was thought to be standardised when the observed values of parachor for standard substances, were in agreement with the standard observed values by other workers, within a limit of 1%. The apparatus was then used for the investigation work.

PART ONE

PARACHOR IN SOLUTION

The first attempt to determine parachor in solution was made by Hammick and Andrew (1929). They used the formula

$$Pm = \frac{Mm\, r^{\frac{1}{4}}}{(D-d)}$$

where Pm is the parachor of the solution and Mm and r the mean molecular wt. and surface tension of the solution. D and d, the respective densities of liquid and vapour. Mm is given by

$$Mm = (1-x)\ Ms + x\ Mx$$

where x and (1 − x) are molecular fractions of the solute and the solvent and Mx and MS their respective molecular wts. Pm was given by

$$Pm = (1-x)\ Ps + x\ Px$$

where Px and Ps are the parachors of the solute and the solvent respectively.

They used several different solvents and solutes associated and non associated both and found that the value of Px was in some cases independent of x and in some others dependent on it, so that the real value of Px could be found by extrapolation of x = 1. With water as a solvent they reported anomalous results. Thus they have shown that the mixture law can be used to find out the parachor of liquids in liquid mixtures.

The same authors applied the mixture law to solid-liquid solutions and came to the conclusion that the parachor of solid in solution is lower by 10 units. Ray came to the conclusion that solid-liquid mixtures obey the straight line mixture law. Bhagwat and co-workers have shown that the variation observed by Ray may be due to experimental error and cannot be due to the applicability of mixture law. In many papers published by Bhagwat and co-

workers, it has been shown that when a solid is dissolved in a solvent, the observed values are in general a little lower than the calculated values and that they are independent of concentration Thus the simple mixture law holds good for them.

The present investigation was undertaken to study the variation in the application of mixture law when the solute is a solid and when it is a liquid. I have taken low melting solids and determined their parachor in pure liquid state by taking measurements above the melting point The solid was dissolved in a suitable liquid and the parachor was determined at temps. below and above the melting point of the substance. It is thus possible to compare the results of parachor for the same substance in a pure liquid state and when it is dissolved as a solid and when it is dissolved as a liquid

A summary of our observations for parachor of some low melting substances in different solvents for different concentrations and temps is given below

Substance	*Observed* *M P C*	*Px below* *M P*	*Px above* *M P*	*Calculated* *Parachor*
Urethane	47	206 6	207 2	209 8
Phenol	42	218·0	224 6	220 0
p-nitrotoluene	52	291·9	293·0	304 0
P-Cresol	35	257 5	257·0	266·1
P-toluidine	43	261 6	273·9	271 4
Benzophenone	42	409·9	425 3	428·0
P-dichlorobenzene	53	262·4	277 1	281 5
L- napthylamine	49	331 4	339·4	342·6
O-nitrophenol	45 4	262·0	273 1	285·0
Menthol	41	433 1	429·4	416 1

Our results indicate that the mixture law is applicable to both liquid liquid and solid-liquid mixture only in case of urethane, phenol p-nitrotoluene and p-cresol. In these cases the parachor values, below and above the melting point, are almost the same and that these values approach the calculated values in case of urethane and phenol while in the case of p-nitrotoluene and p-cresol, the parachor of the substance is low due to association.

However in case of p-toluidine, benzophenone p-dichlorobenzene, l-napthylamine and o-nitrophenol, the mixture law is applicable to liquid liquid mixture but not to solids dissolved in liquid This may be due to greater-association in solid state. The behaviour of menthol is both peculiar and exceptional

In certain cases the calculated values are different from experimental values. The difference may be due to association, ionisation resonance or hydrogen bonding.

My investigation also establishes the point, that concentration of the solution does not affect the parachor of the substance.

Part Two

Hydrogen Bond and Parachor

It is known that under certain conditions an atom of Hydrogen is attracted by rather strong forces to two atoms, instead of only one so that it may be considered to be acting as a bond between them. This is called the hydrogen bond. It is largely ionic in character and is formed only between the most electro-negative atoms The strength of the bond increases with the increase in the electronegativity of the two bonded atoms. The presence of hydrogen bond and it s strength is determined chiefly by studying the bond energy the absorption spectrum in the infra red region or inter-atomic distances by x ray diffraction Other physical properties like volatility solubility and molecular wt. are also found useful.

I have in my thesis, studied the formation of hydrogen bond by parachor method I have attempted to investigate the formation of hydrogen bond in a number of compounds, from their parachor values. Some of these compounds form strong hydrogen bond while others a weak hydrogen bond. I have also attempted to investigate, whether parachor method can be of use in distinguishing a strong hydrogen bond from a weak hydrogen bond.

In calculating the parachor of solute in solution the mixture law of Hammick and Andrew has been used.

A summary of our results of parachor of investigated compounds with different solvents for different concentrations and temperatures is given below

Summary

Substance	*Calculated value*	*Observed value*	*Difference in value*
Compounds Forming Strong Hydrogen Bond			
Methyl salicylate	334·1	323·7	10·4
Salicylaldehyde	275·1	266·5	8·6
O-Nitrophenol	285·7	272·9	12·8
Acetic acid (Dimer)	282·2	260·0	22·2
Propionic acid	360·2	340·0	20·2
Benzoic acid	550·0	537·0	13·0
Compounds Forming Weak Hydrogen Bond			
O-Cresol	266·1	257·3	8·8
O-mono Chlorophenol	265·7	255·2	10·5
Pyrogallol	267·1	257·9	9·2
Resorcinol	24 1	239·2	7·9
Hydroquinone	247·1	237·4	9·7
meta-Nitrophenol	285·7	273·9	11·8
p-Nitrophenol	285·7	273·7	12·0

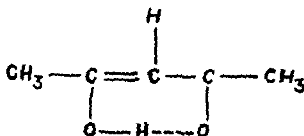
It is observed that when a hydrogen bond is present in the structure of a substance, it's parachor value shows a convincing and sudden drop of about ten units In case alternate resonating structures were possible for these substances then there would be no change in parachor value or the difference in the observed parachor and calculated one would have been small. Thus parachor determination can decide between the resonating and hydrogen bond structures, if both alternate structures are possible.

Our observations also clearly indicate that the parachor values fail to distinguish a strong hydrogen bond from a weak one.

It is thus concluded that the parachor method is inadequate to determine the strength of hydrogen bond.

STRUCTURE OF ACETYL ACETONE

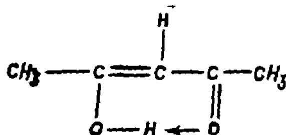
Pauling has mentioned the formation of hydrogen bond in acetyl acetone between the hydroxyl group and the adjacent oxygen atom of the ketonic group. It's structure according to him is as follows



I have determined the parachor of acetyl acetone in benzene, chloroform, carbon tetra chloride, tolune, dioxane and methyl acetate at different concentrations and temperatures and observed a constant value in solution of about 245 5 The calculated parachor is 247 2. Thus the observed values of parachor do not suggest the existance of hydrogen bond

The presence of a co-ordinate bond is indicated by a drop of 1·6 units in parachor Hydrogen atom of hydroxyl group of acetyl acetone is known to be ionic in character being replaceable by metals, resulting in compounds forming a chelate bond. It is possible that this largely ionic character of the hydrogen atom is responsible for the formation of co-ordinate bond instead of a hydrogen bond. Our observed parachor value corresponds with the formation of a co-ordinate bond, assuming that the parachor value of ring formation due to co-ordinate bond is zero as in the case of ring formation due to hydrogen bond.

Therefore the structure of Acetyl acetone should be as follows



Attention may also be drawn to some paper already published (S. G. Khandekar *et al Journ Ind Chem. Soc* 1952, 29 37 1952, 29, 301 1952, 29, 679 and *Agra Univ J Res* (Sci) 1957 1 169 1957 6 53 1957 6, 53) as they throw further light on the subject.

VARIATIONAL PRINCIPLES IN HYDRODYNAMICS AND ALLIED TOPICS*

S. C. Gupta

Mathematics Department Indian Institute of Technology Powai Bombay

The thesis embodies an introductory chapter on the Calculus of Variations and five chapters more. The first contains a review of the various variational principles in Hydrodynamics and concerned topics formulated hitherto. The second chapter presents two variational principles (related with each other) for the scalar wave equation. The third chapter deals with the formulation of two variational principles for convective heat transfer. The first deals with the steady convection of heat in uniform channels and the second with translent heat convection in anisotropic media. Lastly a least square procedure for the variational principle in the anisotropic case is also given. In the fourth chapter three variational principles for viscous incompressible magnetohydrodynamic flow have been given. The first two are restricted and the third a general one. The fifth chapter deals with two specific problems and is not connected with the previous chapters. The first problem is that of a flat plate moving broad side on slowly in a viscous liquid. The second is a result concerning a non linear elliptic partial differential equation in the theory of gas-lubricated bearings.

Introduction

This chapter briefly introduces the subject of the Calculus of Variations. This is required in the following chapters. The chapter starts with the classical problem of extremizing the integral

$$I = \int_a^b F\left(x, y, \frac{dy}{dx}\right) dx \qquad (1)$$

where a and b are fixed quantities and continuously differentiable relations between y and x are admissible. The necessary condition (The Euler Lagrange equation) for the extremum has been derived and sufficient conditions for weak variations have been briefly considered.

Indications have been given that the problem can be generalized in various ways.

In order to consider the case of strong variations the problem of variable end points has been presented briefly and the necessary conditions derived. Sufficient conditions have simply been referred to. The concept of the field of extremals has been defined. The property of the Hilbert's inte

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*Summary of the Thesis submitted to the Agra University Agra for the degree of Doctor of Philosophy in Mathematics.

gral that it is invariant with regard to the path of integration remaining confined to the field of extremals has been proved.

Lastly using the two concepts of the Hilbert's Integral and the field of extremals the Weierstrassian E function in connection with the integral I in equation (1) (when strong variations in y are considered, is defined and criteria for strong maxima and minima have been very briefly described.

The material for this chapter has been borrowed from the bibliography mentioned at the end

Chapter I

REVIEW OF VARIATIONAL PRINCIPLES IN FLUID DYNAMICS, MAGNETO HYDRODYNAMICS AND HEAT TRANSFER

This chapter gives a general and brief review of variational principles formulated by various workers in the following fields.

1 Extrema principles for perfect fluid flow Here extrema principles for perfect compressible as well as perfect incompressible flow have been briefly given.

2 Variational principles for viscous flow Comparatively the variational principles for viscous flow are less in number

3 Variational principles for Hydrodynamic and Hydromagnetic stability The work of Chandrasekhar Woltjer and Frieman and Kulsrud has been presented briefly

4 Variational principles for force free fields in Magnetohydrodynamics Some very interesting results (which have been arrived at in the form of variational formulation) by Chandrasekhar and Woltjer regarding force-free fields in Hydromagnetic systems have been given in the form of three theorems.

5 Variational principles in heat transfer A general and brief review of variational principles in heat transfer (heat-conduction, heat-convection free and forced and heat radiation) has been presented.

6 Variational principle for scalar wave equation A brief account of the variational principle of Leech (which also gives a Lagrangian formulation of the problem by introducing generalized coordinates) is given.

Finally it has been indicated how the work was motivated and what the scope and extent of the work is

Chapter II

VARIATIONAL PRINCIPLE FOR SCALAR WAVE EQUATION LAGRANGE FORMALISM

It is well known that the scalar wave equation occurs in varied branches of physics

A variational principle for the scalar wave equation corresponding to the requirement that the difference between total kinetic energy and potential energy may be as small as possible and using the concept of Hamiltonian density already exists in literature (Methods of Theoretical Physics Vol. I P M. Morse & H Feshbach) The canonical equations for the conjugates are not as simple as those encountered in classical mechanics. The dependence of the Hamiltonian explicitly on the space variables x y z introduces the complexity It is clear that the canonical formalism is not suited for the development of statistical mechanics for phenomena governed by the wave equation in continuous media.

The variational principle of Leech (Classical Mechanics chapter 9 J W Leech, Methuen) is the analogue of the Lagrangian formulation in classical mechanics of discrete particles. In his formulation the generalized coordinates have been taken as functions of space variables as well as time and not of time alone. Using the notation of a functional derivative he expresses the Lagrangian equations of the phenomenon in a form similar to the one in classical mechanics. But these equations are essentially complicated and do not convey further information.

This chapter deals with the formulation of a variational principle based on the concept of generalized coordinates, and is slightly different from that of Leech. We consider generalized coordinates q which are functions of t alone. A vector field $\vec{H}$ and a scalar point function V related to the wave function ϕ* are introduced The variation $\delta\vec{H}$ (depending on δq) leads to the Lagrangian form of equations for q which are identical with the equations in classical dynamics. This procedure has been developed by Biot (Jour Aero. Sci. 24 12, pp 857-873 1957) in his treatment of the transient heat-conduction phenomena. This formulation has a distinct advantage over the principle mentioned earlier because in this case Liouville's theorem of a permanent flow in the phase space can be easily proved. Moreover all the general methods developed in classical statistical mechanics are at once applicable to the wave phenomena The essential point of difference would be that the phenomena may depend on a countably infinite number of generalized coordinates This approach is being followed at I I T Kharagpur India by Joshi and Nigam.

The formulation of the variational principle equivalent to the wave equation is achieved as under

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* The wave equation is $c^2\Delta^2\phi = \partial^2\phi/\partial t^2$ here ϕ is the wave disturbance or wave function.

A vector field $\vec{H}$ a scalar point function V and a variational invariant δD are defined by

$$\phi/c^2 = -\nabla . \vec{H} \qquad (2)$$

$$V = \frac{1}{2} \int_T \frac{\phi^2}{c^2} \, dT \qquad (3)$$

and

$$\delta D = \int_T \frac{\partial^2 \vec{H}}{\partial t^2} \, \delta \vec{H} \, dT \qquad (4)$$

T being the volume where the wave disturbance exists. Also we take

$$\frac{\partial^2 \vec{H}}{\partial t^2} = -\nabla \phi \qquad (5)$$

The variational principle is then stated as

$$\delta V + \delta D = \int_S \phi \, \vec{n} . \, \delta \vec{H} \, dS \qquad (6)$$

where the surface integral extends over the surface S bounding the region T and $\vec{n}$ is the unit inward drawn normal to the element dS of S. The principle can be shown to be equivalent to the wave equation.

The latter part of the chapter develops the Lagrange formulation of the principle Also it gives an alternative variational formulation in the Hamiltonian form Later canonical equations identical in form to the ones in classical dynamics have been derived by defining certain quantities called peudo generalized momenta. This prepares the ground for the development of a corresponding Liouville's Theorem for phenomena governed by the wave equation Some general considerations and procedures have been given together with the introduction of the concept of normal and ignorable coordinates. By way of illustration a one-domensional example (a string fixed at both ends) has been solved by the application of the principle.

Chapter III

VARIATIONAL PRINCIPLES IN CONVECTIVE HEAT TRANSFER

This chapter has been divided into two parts The first part deals with the formulation of a variational principle for convective steady heat transfer in uniform channels. Chambers [*Quart.* J Mech Appl. Math 9 (1956),234] and Rosen [J Chem. Phys. *21 (1953) 1220*] have formulated variational principles for heat conduction in solids and heat convection respectively but they have not been explotted to obtain solutions of specific problems The recent work of Biot [J Aero Sc. *24 (1957) 857*] is an exception to this. He has formulated a variational principle for transient heat

conduction in solids and has successfully applied it to some problems of flight structures Biot has used the concepts of thermal potential dissipation function and generalized thermal force to reduce the equations of heat transfer to equations of Lagrangian type The principle is quite general and embraces a large category of physical phenomena. The physical phenomenon is considered to be represented by a vector field instead of a scalar temperature field. This permits the developments of new methods in heat flow analysis described in detail by him. Another attempt to use variational methods for the study of fully developed laminar heat transfer in ducts assuming the axial gradient of temperature to be constant, is due to Sparrow and Siegel [E.M. Sparrow & R. Siegel Trans Amer Soc. Mech Engrs. 81c 2 (May 1959) 157 167] and the cases of square and sector shaped ducts have been discussed.

In a recent work Nigam & Agrawal [S D Nigam & H.C. Agrawal Jour Math. Mech., Vol. 19 No 6 (Nov 1960) 869-884] have developed a variational principle for transient heat convection in isotropic media and another for steady heat convection in uniform channels The latter principle has been applied to the cases of slug and parabolic flow in a channel with parallel walls for large Peclet numbers. Agrawal (A S.M E Publication Paper No. 60-WA-98, 1960) has applied the same principle for a duct of circular cross section This is a natural extention of the work of Biot. The results are found to be in good agreement with those obtained by exact methods. In the reference given above the axial heat conduction has been neglected and this leads to a simplication of the problem. In the variational formulation of this chapter this restriction has been set aside and a variational principle has been developed for the complete heat convection equation for channel flows The treatment is valid for all Peclet numbers. Lagrangian type of equations have been formulated for the thermal flow field using the concepts of thermal potential, dissipation function and generalized thermal force. The effects of surface heat transfer (by radiation from the surface of the tube) have also been included in the formulation General procedures for obtaining solutions of Lagrangian equations for plug flow where the thermal flow field is linearly dependent on the generalized coordinates have been given. The ideas of normal and ignorable co-ordinates have been introduced along the same lines as in the last chapter

As an illustration the problem of heat transfer for plug flow using normal coordinates has been solved, and it is shown that the solution agrees with the classical solution of the problem.

The equation for steady and forced convection of heat in uniform channels for an incompressible fluid of constant heat conductivity k and heat capacity c per unit volume is

$$k\left(\frac{\partial^2\theta}{\partial x^2}+\frac{\partial^2\theta}{\partial y^2}+\frac{\partial^2\theta}{\partial z^2}\right)+\phi=cU\frac{\partial\theta}{\partial x} \quad x\geq 0 \tag{7}$$

where x axis is parallel to the generators of the tube The tubular surface is given by

$$f(y,z)=0 \qquad \dots \quad \dots \quad (8)$$

U (y, z) is the fully developed laminar velocity in the channel and ϕ is the viscous dissipation function The equation (7) represents a three dimensional steady phenomenon in the three variables x, y, z. We introduce a time-like variable ξ in place of x $(=U_0\xi)$ where U_0 is a typical velocity of the system. Equation (7) becomes

$$\phi+k\left(\frac{\partial^2\theta}{\partial y^2}+\frac{\partial^2\theta}{\partial z^2}\right)=c\nu\,\frac{\partial\theta}{\partial\xi}-\frac{K}{U_0^2}\frac{\partial^2\theta}{\partial\xi^2}\quad \xi\geq 0 \quad \dots \quad \dots \; (9)$$

where $U=U_0\nu$ ν being the dimension-less velocity The equation (9) may be regarded as representing a phenomenon in two dimensions y z varying with ξ. This interpretation helps in the formulation of an equivalent variational principle. The two dimensional operator Δ is defined by

$$\nabla\equiv\vec{j}\frac{\partial}{\partial y}+\vec{k}\frac{\partial}{\partial z}$$

where $\vec{j}$ and $\vec{k}$ denote unit vectors in the directions of y and z respectively. Equation (9) becomes

$$\phi+\nabla\cdot(k\nabla\theta)=c\nu\,\frac{\partial\theta}{\partial\xi}-\frac{k}{U_0^2}\frac{\partial^2\theta}{\partial\xi^2} \qquad \dots \quad \dots \; (10)$$

Define a vector field $\vec{H}$, a thermal potential V and a variational invariant δD respectively by

$$C\theta=-\nabla\cdot\vec{H} \qquad \dots \quad \dots \; (11)$$

$$V=\frac{1}{2}\int\int_A c\theta^2\,dy\,dz \qquad \dots \quad \dots \; (12)$$

$$\delta D=\frac{1}{k}\int\int_A\left(\nu\,\frac{\partial\vec{H}}{\partial\xi}-\frac{k}{cU_0^2}\frac{\partial^2\vec{H}}{\partial\xi^2}+\vec{N}\right)\cdot\delta\vec{H}\,dy\,dz \quad \dots \quad \dots \; (13)$$

with

$$\nabla\cdot\vec{N}=-\frac{\partial\vec{H}}{\partial\xi}\cdot\nabla\nu+\phi \qquad \dots \quad \dots \; (14)$$

The integrals in (12) and (13) extend over a section A of the channel. The variational principle is stated as

$$\delta V+\delta D=\int_C\theta\vec{n}\cdot\delta\vec{H}\,ds \qquad \dots \quad \dots \; (15)$$

the line integral extending over the bounding curve C of the section A of the channel $\vec{n}$ denoting an inward unit normal to an element of C. The principle in (15) can be shown to be equivalent to (10)

In the second part of the chapter use has been made of the concepts of a heat flow vector a thermal potential, a dissipation function and generalized thermal force and a variational principle for transient heat convection in the case of anisotropic fluid media has been developed. The equations governing the thermal flow field have been expressed in the Lagrangian form. The effects of surface heat transfer also have been included later.

Lastly a least square principle has also been given for the variational principle of Part II. This gives a method by which the order of accuracy of solutions in various cases can be estimated.

Chapter IV

VARIATIONAL PRINCIPLES IN MAGNETOHYDRODYNAMICS

This chapter deals with the formulation of variational principles for viscous incompressible hydromagnetic equations. The flow is governed by seven equations: the equation of continuity, three equations expressing the interactions of the magnetic field with the velocity field (modified Maxwell's equations) and three equations of motion (modified Navier—Stokes equations). The magnetic field has to satisfy a supplementary condition that it is divergence free. This is not an independent condition but is a direct consequence of the equations of electromagnetism.

Three variational principles have been formulated: two restricted principles and one general principle. In the first restricted variational principle the equation of continuity and the equations of motion are taken as admissibility conditions and the Euler Lagrange equations of the principle turn out to be the three equations showing the interaction of the magnetic field and the velocity field (modified Maxwell's equations).

In the second restricted variational principle the equation of continuity and the three equations showing the interaction of the magnetic field and the velocity field are taken as admissibility conditions and the Euler Lagrange equations of the principle come out to be the three equations of motion of magnetohydrodynamics. In a way both of these principles can be regarded as supplementary to each other. For apart from the equation of continuity the Euler Lagrange equations of one principle are the admissibility conditions for the other and vice-versa. It is assumed that the quantities involved satisfy all the conditions with regard to continuity and differentiability (unless otherwise stated) so as to enable the formulation to be possible. The spatial region within which the fluid motion is considered is of such a character that the application of Gauss Theorem is justified.

The third variational principle is a general one and does not assume any one of the seven equations as admissibility conditions. Use has been made of seven new auxiliary variables (apart from the physical variables) in forming

STUDIES ON THE PATHOLOGY OF PNEUMONIA AND ASSOCIATED PULMONARY DISEASES OF CATTLE AND BUFFALOES*

JAGDISH NARAIN DWIVEDI
Department of Pathology & Bacteriology
U P College of Veterinary Science & Animal Husbandry Mathura (India)

Investigations on the pathology of pneumonia and associated pulmonary diseases in cattle and buffaloes were undertaken to study the gross and histopathological variations in pneumonia, specially to find out the pulmonary lesions in subclinical stages alongwith the occurrence of the lesions of thoracic form of tuberculosis. The possibilities of the hitherto unrecorded infections and other associated conditions of lungs and pleura were also explored in the animals slaughtered at various abattoirs of Uttar Pradesh, India.

The histopathological studies were conducted on the samples of 250 cases collected from the autopsies performed in the department of Pathology and Bacteriology and 1089 animals examined at five abattoirs.

The gross involvement of pneumonic lesions varied considerably in nature and distribution The consolidated pneumonic lesions were observed which were distributed in cardiac, apical and diaphragmatic lobes of 20 cases over cardiac and apical lobes of eight cases and in cardiac lobe of only three cases. The rest of the pneumonic cases showed nodular patchy lesions distributed irregularly througout the various lobes of both the lungs

The lesions on microscopical examinations were categorised under following major heads having similar picture. The lesions of acute bronchopneumonia were observed in eleven cases dying either due to pneumonia or complicated with pneumonia. The lesions similar to subacute and chronic bronchopneumonia were seen in twelve and seven cases respectively Whereas acute suppurative lesions in lungs were much more common and were seen in twentythree cases, the chronic process of suppuration with slight degree of encapsulation was studied in 6 cases

The mixed-granulomatous lesions with actinomycotic bodies at the centre in a single case and bodies suggestive of actinobacillosis in three cases were recorded in the lungs of buffaloes. Septate, filamentous branching hyphae morphologically similar to *Aspergillus* sp alongwith other hyphae probably those of *Nocardia* or *Streptomyces* were observed in a case of mycotic pneumonia with suppurative lesions

* This is an abstract of the Thesis submitted in partial fulfilment of the requirement for the Degree of M. V Sc (Vet Sc) in Pathology of the Agra University in 1961

Multiple small caseous nodules in the lungs of a buffalo histopathologically revealed mixed granulomatous inflammatory reaction with the organism at centre. The microorganisms, because of the difference in size and staining reaction with Haematoxylin and Eosin stain the presence of large nucleus, the Periodic Acid Schiff positive material in some of the cells, the cyst like structure of the some of the cells in necrotic area and the loose cavity surrounding them appeared to be amoeba probably *Acanthamoeba*. This may probably be the first report of such lesion associated with *Acanthamoeba*.

Tuberculous lesions involving lungs, bronchial as well as mediastinal lymphnodes were observed in 16 animals.

In four specimens from the buffalo-lungs, mature flukes characterised by prominent cuticular spines, dendritic gonads and branched intestinal caeca were found embedded in dark brown consolidated nodules with varying amount of fibrosis and haemorrhages around the lesions. These flukes were identified as belonging to *Fasciola gigantica* and has been recorded for the first time in India.

A case of ossification with three cases of alveolar wall calcification in the lungs is also reported for the first time in Indian cattle and buffaloes. Cystic conditions of lungs were common and affected 21·6% of animals examined during the study and caused the replacement of the normal parenchyma of the lungs Foreign bodies like needles were also recovered from two cases.

Lymphosarcoma at the pleura of eleven buffaloes having nodular lesions and histopathologically masses of aggregated lymphocytic cells in varying sizes with congested capillaries and scanty stroma, were studied. In one case only the subpleural lung tissue was infiltrated with lymphocytic cells. The thoracic lymph nodes of two cases were examined and revealed large pale germinal centre and cells resembling lymphoblasts. The nodules were separated by the slight amount of connective tissue and capillaries.

The occurrence of various pneumonic changes tuberculous lesions in lungs and lymph nodes and lymphosarcoma at the pleura alongwith other pathological manifestations, as described above, in slaughtered animals clearly indicates the necessity of undertaking further extensive work on the above problems

STUDIES ON OCCURRENCE OF SALMONELLA SEROTYPES IN PIGS IN RELATION TO PUBLIC HEALTH*

B. D MAKHOLIA

Department of Pathology & Bacteriology

U P College of Veterinary Science & Animal Husbandry Mathura

The present investigation was conducted to find out the occurrence of salmonella species in pigs and to isolate salmonella species from human beings simultaneously from same areas from where pigs were examined It was hoped that such a study would not only reveal the occurrence of salmonella serotypes in pigs but would also be helpful in elucidation of its epidemiological and public health importance.

From pigs 780 faecal samples 68 samples of mesenteric lymph nodes and 77 samples of internal organs were examined The samples were collected from different localities of Mathura villages nearby Mathura, Vrindaban and Central Dairy Farm Aligarh. In case of human beings a total of 347 faecal samples were examined which included 11 samples from clinical cases of diarrhoea These samples were collected from certain localities of Mathura Civil hospitals at Mathura and Vrindaban and hostels of Veterinary College, Mathura.

Kauffmann's modification of tetrathionate broth was used as enrichment medium in combination with brilliant green agar as selective medium throughout the course of this study Modified MacConkey's medium (Lactose replaced by Mannitol) used for purification of colonies from brilliant green agar plates was helpful in purification as well as differentiation of salmonella and proteus colonies.

Salmonella genus specific O I phage and polyvalent serum (A to 53) were used for preliminary identification of cultures By following this procedure indentification of salmonella was considerably hastened and at the same time it was helpful in isolation of larger number of salmonella strains than could have been possible by following routine biochemical tests. On the basis of 'O 1 phage lysis and polyvalent serum agglutination 125 cultures were isolated and 96 of them proved to be salmonella on testing biochemically Ninetythree cultures out of 96 were positive to both phage and polyvalent serum tests one culture was negative to phage lysis and two cultures were negative in agglutination with polyserum Both 'O 1 phage and polyvalent serum gave a number of nonspecific reactions. Fourteen cultures were encountered in this study which were giving positive reactions

*This is an abstract of the Thesis submitted in partial fulfilment of the requirements for the Degree of M. V Sc In Bacteriology of the Agra University in 1962

with both phage and polyvalent serum but did not prove to be salmonella on testing biochemically It may be concluded that by using both 'O 1 phage' has and polyvalent serum agglutination tests the chances of missing any salmonella culture are very rare. But all cultures positive to these tests may not be salmonella.

Biochemical tests though lengthy were necessary for confirmation of the cultures isolated on the basis of two tests mentioned above, as well as for elimination of cultures giving nonspecific reactions. A number of biochemical varients were encountered. One culture which was fermenting both lactose and salicin proved to be salmonella, such cultures have very rarely been reported.

Somatic and flagellar antisera were prepared for use in serological typing. Both slide and tube agglutination tests were performed in typing of cultures.

In case of pigs 50 salmonella strains were isolated which included two strains from mesenteric lymph nodes and three strains from pooled internal organs From human beings 46 salmonella strains were recorded including 9 strains from clinical cases. In a total of 96 salmonella strains following 20 serotypes were encountered.

Name of Serotypes	Strains from pigs	Strains from man	Total
S anatum	5	6	11
S. bareilly	7	9	16
S bovismorbificans	1	6	7
S chester	2	5	7
S cubana	1	2	3
S dublin	1	2	3
S newport	6	1	7
S stanley	3	2	5
S typhimurium	4	3	7
S weltevreden	10	7	17
S worthington	1	1	2
S. vrindaban (New Type)	1	—	1
S. concord	1	—	1
S london	1	—	1
S magwa	1	—	1
S. poona	3	—	3
S richmond	1	—	1
S. sandiego	1	—	1
S dakar	—	1	1
S tennessee	—	1	1
Total	50	46	[illegible]

As is evident from the above table eleven serotypes were isolated both from pigs and human beings seven species including the new type *S. wandsbek* were recorded only from pigs Two species *S. dakar* and *S. tennessee* were isolated only from human beings This is the first record of these *two species in India. The importance of epidemiological relationship* of occurrence of salmonella in pigs and human beings is discussed.

A STUDY OF VARIATIONS IN THE PHOSPHOLIPIDS OF MILK AND THEIR PARTITION IN MILK PRODUCTS*

RAJE SINGH RAWAT
S K N Govt College of Agriculture Jobner (Jaipur)

SUMMARY

Phospholipids are the normal constituents of every living cell. Their discovery dates back to the early part of the eighteenth century although that milk also contains phospholipids was recognised as late as 1900

There is sufficient evidence to show that the field of phospholipid study has been subjected to intensive investigation by numerous scientists in many parts of the world and considerable amount of scientific knowledge has been gathered in respect of the role of phospholipids in various biochemical and physiological processes of life. Nevertheless one finds serious conflicting reports on the phospholipid content of milk on the one hand, and numerous gaps in our knowledge of the partition of these compounds in milk products on the other as a result of various natural and technological factors. This formed the basis of the present investigation which aims at studying the variation in the phospholipid content of milk and their partition in milk products

The details of the programme of this investigation are as under —

I Standardisation of the technique in respect of —

(a) The extraction of phospholipids from milk and milk-products.
(b) The digestion of the phospholipid extract.
( ) Quantitative estimation of phospholipid in milk and milk products

Prior to anything else the steps involved in the use of Klett-summerson Photoelectric colorimeter for the estimation of phosphorus were checked and set.

II. Studies on the variation in the phospholipid content of milk due to :—

(a) Species.
(b) Breed.
(c) Individuality
(d) Stage of Lactation.
( ) Udder disorders
(f) Season
(g) Feed,
(h) Processing of milk.
(i) Ageing of milk.

*Summary of the Thesis submitted for the degree of Doctor of Philosophy of the Agra University in 1962.

III Studies on the variation in the phospholipid content of curd as influenced by :—

(a) Heat treatment given to milk.
(b) Acidity development in the curd.

IV Studies on partition of phospholipids :—

(1) Partition of phospholipids of milk between cream and separated milk as influenced by —
    (a) Ageing (b) Agitation of milk.

(2) Partition of phospholipids of cream between butter and buttermilk as influenced by —
    (a) Acidity of cream (b) Washing of butter

(3) Partition of phospholipids of milk into butter and buttermilk in the indigenous method of butter making.

V Studies on the transfer of phospholipids of butter to ghee or dry butterfat as influenced by the following methods of manufacture —

(1) Indigenous butter or curd butter method —
    (a) Direct-boiling-off process.
    (b) Pre-stratification process.

(2) Cream butter method —
    (a) Direct boiling-off process.
    (b) Pre-stratification process.

Besides these two methods of manufacture the stage of straining was also studied alongside

VI Variations in phospholipid content during storage of butter and ghee.

(1) Studies on the effect of metallic contamination on the phospholipid content of butter during storage at 40°F
(2) Studies on the effect of oxygen concentration on the phospholipid content and storage life of ghee stored at room temperature (60° 90°F)

The results of this study and the conclusions drawn are summarised below —

I. *Standardisation of the technique*

1 In standardising the technique of colour estimation for the determination of phosphorus in Klett-summerson-Photoelectric Colorimeter it was found that consistent colorimeter readings could be obtained upto 15 to 20 minutes with aminonaphthol sulphonic acid stored upto 7 days in the refrigerator after which period the reagent had to be prepared afresh

2 The Roese-Gottlieb method of extraction gave more consistent results than the Alcohol-ether extraction method. Extraction with alcohol-ether was found to be time consuming Long time was required to evaporate the extract, sometimes 3 to 5 hours which affect the results due to the insolubilisation of phospholipids in petroleum ether ultimately resulting in inconsistent results.

3 In digestion of organic matter with perchloric acid it was observed that occasional shaking hastened the process of d gestion and spattering and explosions were avoided.

4 Large quantities of perchloric acid was required to digest extracts containing excess of fatty matter in the case of butter and ghee or dry-butterfat as compared with the quantity of perchloric acid required in the digestion of the extract from milk. It was found necessary to neutralize the digested material and then add 1 2 ml. of perchloric acid to the material prior to the addition of reagents for the colorimeteric estimation of phosphorus

5 In the elution of individual phospholipids it became essential to wash the MgO twice with petroleum ether instead of washing once only as in the latter case the MgO-methanol mixture did not separate clearly when centrifuged for the adsorption of the phospholipids on MgO

6 It was found that the hydrolysate containing the acid soluble phosphorus (lecithin P) when digested with perchloric acid formed a white precipitate, which increased the colorimeter reading In the estimation of lecithin P it was, therefore necessary to centrifuge the material to settle the insoluble matter before taking the reading in colorimeter

II. *Phospholipid content of Milk*

1 Species variations were prominent. The total phospholipid content of cow buffalo goat and sheep milk was 30 15 36 90 43 85 and 43 04 mg per 100 ml. respectively which correspond to 0 0301 0 0369 0 0438 and 0 0430 per cent wt./vol On fat basis, the values were found to be 0.619 0.505 0.933 and 0 740 per cent for cow buffalo, goats and sheep respectively Species variations were also found in the component phospholipids. The ratio of lecithin cephalin, and sphingomyelin was 48 40 12 in cow 40 48 12 in buffalo 48 37 15 in goat and 48 39 13 in sheep milk.

2. Variations due to breed were not so wide as due to species The total phospholipid content of the milk of Hariana and Sahiwal cows were 31.61 and 29.90 mg per 100 ml respectively

3. Wide variations were observed in the phospholipid content of milk from individual animals in the same breed The total phospholipid content of milk from Hariana cows varied from 14 72 to 39 72 mg per 100 ml. and from 0.3978 to 0.6917 per 100 gm. fat.

4 The stage of lactation of the animal produces significant variations in the phospholipid contents. Colostrum from Murrah buffaloes had on an average 60.32 mg of total phospholipids per 100 ml., which reached the normal value in 7 days after parturition On fat basis the value ranged from 0.5668 to 1.2814 per cent.

From first month of lactation to the sixth month of lactation the average value for total phospholipids in milk ranged from 33·71 to 39·8 mg per 100 ml. From seventh month onwards upto the end of lactation there was considerable increase in the phospholipid content of milk. The increase is attributable to the increase in the fat content and the decrease in the size of fat globules.

5 Definite increase in the phospholipid content of milk was observed due to udder disorder The average total phospholipid content of milk from normal quarter were 36·49 mg per 100 ml milk and 0.830 gm. per 100 gm. fat From abnormal quarter of the same animal the average values were 56 16 mg per 100 ml. milk and 1 33 gm. per 100 gram fat.

6 Season has been found to affect the phospholipid content in cow milk. Lower values in summer and higher values in winter months were recorded. Seasonal variations have been attributed to seasonal nature of calvings, most of the cows calving in February and March

7 The phospholipid content of milk was high when the animal were getting dry fodder and low when they were getting plenty of green fodder

8. No significant effect of processing was observed on the phospholipid content of milk. However an increase in the phospholipid content of heat treated milk was found to be due to the concentration of the milk and the consequent increase in fat per cent.

9 No significant effect on the phospholipid content of milk has been observed during storage for 12 hours

III *Phospholipid content of curd*

In studying the variation in the phospholipids of curd, it was observed that there was no effect either of heat treatment given to milk or of acidity development in the curd

IV *Partition of Phospholipids*

1 Partition of phospholipids of milk between cream and separated milk was found to be affected by ageing and agitation of milk prior to separation.

The total phospholipids in cream (47 89 per cent fat) from fresh milk were 0 1579 and in cream (46 17 per cent fat) from milk stored for 14 hours

were 0·1553 per cent. On fat basis the values for the corresponding samples were 0·3680 and 0·3365 per cent

In separated milk the total phospholipids were 0.0124 and 0.0140 per cent from fresh milk and from stored milk respectively and on fat basis the values were found to be 22·214 and 17.587 per cent respectively

In comparing the effect of agitation of milk on the partition of phospholipids between cream and separated milk, it was found that when the milk was put to sufficient agitation before cream separation, a much larger proportion of phospholipids went with separated milk.

Cream (39·43 per cent fat) from unagitated milk contained 0·1488 per cent, and cream (46·17 per cent fat) from agitated milk contained 0 1210 per cent total phospholipids. On fat basis the values for the corresponding samples were 0·3768 and 0·2763 per cent respectively

Similarly separated milks from unagitated and agitated milks contained 0·0095 and 0·0162 per cent total phospholipids respectively On fat basis the corresponding values were 23·00 and 36·04

2. In a study on the partition of phospholipids of cream between butter and buttermilk, it was observed that acidity of cream prior to churning, and washing of butter affected the partition of phospholipids of cream

On churning sweet cream 40 per cent of the phospholipids went with butter while this amount dropped to 33 per cent in case of sour cream.

The total phospholipid contents on fat basis were found to be 0·1516 per cent in butter from sweet cream (0 22 per cent serum acidity) and 0·1265 per cent in butter from sour cream (0·65 per cent serum acidity) The results suggest that sweet-cream butter will have greater stability against oxidative deterioration.

Samples of butter obtained without washing, with one washing, and with two washings contained 0·2464 0·1743 and 0·1265 per cent total phospholipids on fat basis The results therefore suggest that too much washing of butter affects the phospholipid content of butter which may in turn affect the keeping quality of the product

3 In a study on the partition of phospholipids of milk between butter and buttermilk in the indigenous method of butter making it was found that of the total phospholipids present in milk 35 per cent went with butter and 60 per cent remained in butter milk, and about 5 per cent are lost during washing and other operations.

The total phospholipids in desi butter and buttermilk were found to be 0·1834 and 0·0162 per cent respectively On fat basis the values for the corresponding products were 0·2093 and 21 57 per cent.

V *Transference of Phospholipids to Ghee*

1 The method of clarification of butter into ghee has been found to affect the phospholipid content of ghee.

2 Ghee from cream butter obtained by direct-boiling-off process contained 0 136 per cent and that obtained by pre-stratification process contained 0·0938 per cent total phospholipids

Similarly from curd butter ghee obtained by direct-boiling-off process contained 0·089 per cent and that obtained by pre-stratification process contained 0 078 per cent total phospholipids.

3 Both in the case of cream-butter and curd-butter methods of ghee making ghee obtained by direct boiling-off process contained higher phospholipid content than ghee obtained by pre-stratification process.

4 The results suggest that other things being the same ghee with greater stability against oxidative deterioration can be obtained by direct-boiling-off process

5 It has been observed that ghee or dry butterfat from the same lot of butter clarified under identical conditions contained more phospholipids when filtered hot (70°-80°C) than when filtered on cooling The results suggest that by filtering ghee while still hot, ghee with superior resistence to oxidation can be produced.

VI *Phospholipids and storage of butter and ghee*

1 Phospholipids of butter and ghee have been found to decrease during storage period.

2. On a study of the phospolipid content of butter during storage period of 120 days, it was found that iron and copper contaminations catalysed the decomposition of phospholipids of butter

3 During a storage period of six months, it was observed that there was little effect on the phospholipid content of ghee stored without any head space in the container and of ghee stored on vacuumisation.

4 Higher concentrations of oxygen and packing with carbon-di-oxide affected the phospholipid content of ghee greatly and reduced the induction period of the product This leads to the conclusion that storage of ghee with container full to the brim does not affect the phospholipid content and increases the keeping quality of the product.

5 It was further observed that the induction period of ghee during storage is affected when the phospholipid content falls below 0·07 per cent.

STUDIES ON PATHOLOGY OF CHRONIC RESPIRATORY DISEASE OF POULTRY*

BALWANT SINGH*

Department of Pathology & Bacteriology
U P College of Veterinary Science & Animal Husbandry Mathura (Ind)

Studies on pathology of chronic respiratory disease CRD) were undertaken to find out the various pathological changes and the degree of involvement n naturally infected cases and to correlate them with cultural and serological findings wherever possible and to conduct the pathogeni trials in chicks from pleuropneumonia like organisms (PPLO) free flock.

The present study was conducted on tissues obtained from various sources such as 2943 field cases under three months of age and 358 over three months from Poultry Farm Mathura and some other places 355 cases sacrificed at 147 days of age from the Department of Animal Genetics and Breeding, 75 cases from experimental birds kept in the Department of Pathology and Bacteriology

The sections were stained with usual haematoxylin and eosin stain for routine histopathological examination and Periodic Acid Schiff Maccalum Good Pasture s stain and Jenner's Giemsa stain were applied only wherever necessary The gross pathological changes ranged from typical gross lesions of CRD to no lesions

The histopathological examination of cases having typical gross lesions revealed lymphofollicular foci and giant cell granulomas n lungs and air sacs diffuse mono-nuclear infiltration n trachea and air-sac vacuolar degeneration and tuboalveolar elongation of intraepithelial glands of trachea These lesions were found to be variable in extent and degree of involvement Similar microscopic lesions were observed in 52 cases out of 826 which did not show any gross lesions.

The histopathological examination of 355 cases sacrificed at 147 days of age showing no gross lesions revealed lymphofollicular lesions in lungs and/or trachea in 130 cases. The vacuolar degeneration and tuboalveolar elongation of mucosal glands of trachea in 90 cases and diffuse lymphocytic infiltration in tracheal submucosa of 60 cases were noticed. The PPLO were isolated from 225 cases and 68 cases were positive for PPLO agglutinins Lymphofollicular lesions in lungs and/or trachea were found in seven out of

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* Present address Department of Pathology and Bacteriology Punjab College of Veterinary Science and Animal Husbandry Hissar Punjab

nine birds obtained from Poultry Farm, Mathura and maintained at the Department. PPLO were isolated in seven birds and serologically only two were positive. No significant gross or microscopic lesions were observed in 670 dead in shell embryos.

The results of experimental studies conducted on PPLO free chicks indicated the desirability of carrying out further studies.

The implications of gross and histopathological findings and their correlation with cultural and serological examination is discussed. The necessity of carrying out further studies and the desirability of raising the PPLO free flocks are stressed

STUDIES OF ELECTRICAL AND MAGNETIC PROPERTIES OF THIN EVAPORATED FILMS OF COPPER SILVER AND GOLD

V V SHAH
Gujrat College Ahmedabad.

SUMMARY

The present work is undertaken with a view to studying the electrical and magnetic properties of thin films of copper silver and gold prepared by thermal evaporation of metals under vacuum. The work is directed to study in a systematic way the following important properties :—

(i) Study of the variation of electrical resistance of thin films on annealing and calculation of the activation energies for the migration of point defects from the observed changes of resistance with temperature

(ii) Study of the variation of electrical resistivity and temperature coefficient of resistance with thickness of the films

(iii) Study of the variation of Hall coefficient with thickness and magnetic field.

INTRODUCTION

Sir J J Thomson[1] was the first to explain theoretically the observed high resistivity of thin films, on the assumption that the scattering probability of the conduction electrons increases when the dimensions of the conductor become comparable with the mean free path of the electrons. Later on Planck[2] proposed an empirical formula relating the thickness to the resistivity of thin films. Other formulae have been proposed by Lovell[3] Appelyard and Lovell[4] and Weale[5] An important contribution in this field was made by Fuchs[6] and Dungle[7] and a comprehensive review of the subject is given by Sondheimer[8] More recent work on vacuum deposited films by Story and Hoffman[9] Neugebaur[10] Buckel[11] etc. indicates that a substantial contribution to the observed high resistivity of thin films is due to the presence of lattice defects (such as vacancies interstitials, dislocations etc.) Further it has been found that metal films deposited in vacuum are in a state of high tensile stress

The experimental study of Hall coefficient in thin films is complicated because of the non-uniformity of the film surface and lack of accurate knowledge of thickness. A theoretical treatment of the Hall coefficient in thin films is given by Sondheimer[12] Steinberg[13] observed a low value (compared to the bulk metal value) of Hall coefficient in thin films of copper and silver

This is a summary of the thesis submitted and approved for the Degree of Doctor of Philosophy of the Agra University in 1962.

Bonfiglioli et al[14] working on gold films found a maximum value of Hall coefficient at about 300 A U thickness The work of Corkler[15] on evaporated films of potassium indicates that the Hall coefficent increases with decreasing thickness upto 50 A. U thickness, and below this thickness there is a steep fall

EXPERIMENTAL

An evaporation plant was designed and constructed for the preparation of thin films, with provision for heating the films under vacuum and at the same time measuring their resistance and temperature. The heating process helps in annealing out the imperfections and making the film free from tensile stress The measurement of resistance under vacuum is preferred, since the properties of the films, when exposed to the atmosphere, are affected due to the adsorption and chemisorption of gas molecules by the surface of the film.

The films were deposited at room temperature, on 'Gold seal' microscope slides by evaporating spectroscopically pure metals in a vacuum of nearly 10^{-6}mm of Hg., and using a liquid air trap The substrates were cleaned, first, by chemical methods as suggested by Strong[16] and finally by ionic bombardment and baking process (upto 200°C) under vacuum. The Substrate distance was always 15 cms from the evaporating source (a molybdenum boat) A high vacuum metal cock was introduced between the vacuum chamber and the diffusion pump to avoid oxidation of the diffusion pump oil during the initial stages of evacuation. The motions of the supporting rods for the furnace, discharge probe, thermocouple etc. were controlled by the use of Wilson seals

While annealing the films under vacuum, the resistance and temperature were recorded at regular intervals of time The resistance was measured with a Kohlrausch bridge in conjunction with a sensitive galvanometer (10^{-9} amp /div) and the temperature with a thermocouple placed in contact with the film

After heating the films they were taken out of the vacuum chamber and their D C. Hall coefficient was measured with a precision Pye potentiometer (reading upto a micro volt) in conjunction with a multiflex galvanometer (10^{-9} amp /div) The primary current through all i.e films studied, was nearly 40 milliamperes and the field was varied from 800 to 2194 gauss Care was taken to eliminate the errors due to the presence of misalignment voltage, the Ettingshausen effect, the Nernst effect and Righi-Leduc effect by using the current and potential leads of the same metal as the film, and by taking four observations (for four different combinations of current and field directions) for a given current and field strength. Keeping in view the effect of finite length of the Hall sample, the ratio, length to width for each film was maintained at nearly three.

The film thicknesses were determined by optical and electrical methods. The optical method of measurement of thickness is based on the principle of Fizeau Fringes (Tolansky[17]) and the electrical method on the theory of electrical conductivity of thin films developed by Fuchs and Sondheimer However with the optical method it was not possible to measure thicknesses below 100 A. U and hence the thickness values obtained from the electrical conductivity method were used for all the calculations.

RESULTS AND DISCUSSIONS

Resistance change before and during heating

All the films studied indicated a rapid fall of resistance just after deposition. A high percentage of this decrease was found to occur within first few seconds of deposition and then the decrease continued slowly for about an hour at the end of which the resistance was steady A further decrease in resistance with increasing temperature was observed when the films were heated inside the vacuum chamber which continued till a minimum resistance was attained by the film at a particular temperature The temperature was not allowed to rise beyond this value so as to avoid formation of agglomerates and discontinuity in the film

The early decrease in resistance suggests that in the process of deposition of the film a large number of interstitials (together with other imperfections) are trapped in it and these being unstable and highly mobile at ambient temperatures, disappear immediately This view is supported by the experimental and theoretical results obtained by several workers (Van Bueren[18] Mott and Gurney[1] Seitz and Huntington[20] Meshi and Kauffman[21] etc.)

The heat treatment under vacuum, removes many other defects (such as vacancies) which migrat to the surface or sinks (probably dislocations) Vand[22] has developed a theory to measure the activation energy for the migration of such defects. The calculated values agree reasonably well with the theoretical results obtained by Dexter[23] for the migration of vacancies in noble metals. This agreement suggests that during the process of heat treatment, the decrease in resistance is due to the disappearance of vacancies

The calculations further indicate that the concentration of defects in thin films is very high compared to that, observed by several workers on quenched bulk noble metals (Meshil and Kauffman[21] Piercy [4])

The experimental results for the variation of resistivity with thickness indicate that in the region of very small thicknesses the resistivities of thin films is nearly 7 to 9 times the bulk metal value and approaches that of the bulk, as the thickness increases However the resistivities, even for the thickest annealed films, are found to be higher than the bulk metal value. This

is generally due to (i) the difference in the texture of the film and the bulk metal, (ii) the presence of unannealed imperfections and impurity atoms and (iii) the size effects.

The temperature coefficient of resistance (TCR) of the films was calculated using the dR/dT values from the reversible linear part of the resistance temperature curves and was found to be positive for all the films studied. In the past several workers have quoted negative TCR for thin metallic films. However it should be remembered that the measurement of the TCR from the irreversible part of the resistance temperature curve is meaningless since in this region, the annealing out of defects gives rise to a negative change of resistance which obscures the true TCR of the material of the film.

The results of the present work indicate that the TCR in thin annealed films does not remain constant, in the range of thickness studied, but varies with thickness and shows maximum and minimum values at particular thicknesses. The maximum values of TCR for Cu, Ag and Au are respectively at 155, 350 and 324 A. U thicknesses and roughly 10 3 and 4 5 times higher than the corresponding bulk metal values. The minimum values of TCR are nearly 0 5 0 3 and 0·6 times lower than the bulk values at film thicknesses 38 74 and 39 A. U for Cu Ag and Au respectively

This type of behaviour of TCR in thin films may be due to the size effects playing some important role and leading to relatively different changes in the values of dR/dT and resistance in different regions of thickness.

Hall effect in thin films

The Hall voltage for each film studied, is found to increase linearly with different slopes in two different regions of the magnetic field (i) 0·8 to 10 KG and (ii) 14 to 23 KG In the second region of the magnetic field, the linear portion has comparatively larger slope than that in the first region Exactly similar behaviour is observed for the variation of Hall angle with magnetic field.

The experimental results for the variation of Hall coefficient (A_H)* with thickness indicate that A_H first increases with increasing thickness reaches a maximum value at a particular thickness and then again decreases. The maximum values of A_H are observed at 155 368 and 222 A.U thickness for Cu Ag and Au films respectively Compared to the standard bulk metal values the maximum value of A_H for copper is 31 per cent higher and those of Ag and Au are lower respectively by 10 and 21 per cent. In the case of copper excepting the maximum value all other values of A_H for different films are lower compared to the standard bulk metal value.

For the field dependence of Hall coefficient in thin films the results of the present work indicate that, in general A_H is maximum at 5 KG, minimum

* A_H represents Hall Coefficient in thin films.

at 15 KG and beyond this field strength it again increases However for comparatively thinner films of copper (38 54 69 A. U thickness) A_H did not show any maximum or minimum but increased slowly with increasing H Similarly for the gold film of thickness 417 A. U thickness A_H remained constant upto 15 KG and then it was found to increase with increasing magnetic field.

Experimental results and Sondheimer's theory

For the thickness dependence of A_H Sondheimer's theory predicts that in the limit of vanishingly small magnetic fields A_H should increase above the bulk metal value as the film thickness decreases This is not in agreement with the experimental results given above

Similarly for the field dependence of A_H theory predicts that the Hall coefficient for different thickness should not vary very much for comparatively small magnetic fields but should approach the bulk metal value at very large fields of the order of 10^8 to 10^9 gauss This is again in disagreement with the experimental results mentioned above

This disagreement may be due to the departure of the experimental conditions from those of ideal ones assumed in developing the theory For example Sondheimer's theory assumes a massive metal film having perfectly plane and parallel surfaces In actual practice, these conditions are not realised for the evaporated films as has been suggested by several workers (Pippard[35] Heavens[36]) and hence the experimental results are bound to differ from those of ideal films.

In the second place Sondheimer's theory assumes that the conduction electrons are quasi free and a time of relaxation can be defined for their collision with the ionic lattice of the metal These considerations give rise to a finite magneto-resistance and field dependence of Hall coefficient in thin films. However the origin of these effects in thin films is different from that of such effects in bulk metals The recent results on skin effect in copper (Pippard[37] and the de Haas-van Alphen effect in copper silver and gold (Shoenberg[38] strongly suggests that in these metals the free electron model is not valid Further the magneto-resistance results obtained by Alekseevski and Gaidukov[39] and Gaidukov[40] for copper silver and gold single crystals are consistent with the shape of the Fermi-surfaces of these metals proposed by Pippard and Shoenberg

To sum up we may say that the assumption as regards the validity of free electron model in noble metals is not fully justified.

The two-band theory (Jan[41]) and the experimental results

The two-band theory of bulk metal for the variation of Hall angle (or Hall voltage) with magnetic field predicts that the Hall angle should increase

BOLBITIS EGENOLFIA AND RELATED FERNS*

SURJIT KAUR
National Botanic Gardens Lucknow

The phylogeny and relationships of the genera *Bolbitis* and *Egenolfia* (Lomariopsidaceae) are subjects of free disagreement between various Pteridologists. The morphology of these genera as well as that of the majority of the ferns with which relationships have been suggested is little known and no two authors agree on the hypotheses of their possible affinity. Suffice it to mention that even as late as 1941 eminent Pteridologists like E. B. Copeland have strongly supported a Polypodioid affinity of the Lomariopsidoid ferns claiming close relationship to such ferns as *Phlebodium*. Most authors prominent among whom are Carl Christensen (1938) and R. C. Ching (1940) have been content to regard these as Aspidioid genera of doubtful relationships. Lately Copeland (1947) has revised his opinion regarding these ferns, contending that they are of Dryopteroid rather than Polypodioid affinity. According to Bower (1928) *Bolbitis* and *Egenolfia* are probably derived from Thelypteroid ferns like *Meniscium* and *Goniopteris*. On the other hand Holttum (1947, 49, '54) considers them to be evolved from a Dennstaedtioid stock and has separated them from Aspidioid ferns as a separate subfamily Lomariopsidoideae.

The present study embodies the result of investigations on the morphology of the sporophytes and gametophytes of all the Indian species of *Bolbitis* and *Egenolfia*. For comparison the morphology of the Dennstaedtioid and Dryopteroid ferns with which relationship has been suggested by various Pteridologists (*Dennstaedtia, Hypolepis, Microlepia, Acrophorus, Diacalpe, Lithostegia* and *Peranema*) are studied.

The rhizome is creeping, slender and branched in *Bolbitis*, *Egenolfia* and the Dennstaedtioid ferns; it is erect, stout and unbranched in the Dryopteroid genera. Basally attached gland-tipped paleae cover the rhizome except in *Dennstaedtia elwesii* and *Microlepia* in which the rhizome is hairy. Glandular hairs occur marginally on the paleae in *Bolbitis* and *Egenolfia* and both marginally and superficially in the Dryopteroid ferns. The paleae originate as uniseriate hairs in which the intercalary cells undergo longitudinal divisions resulting in broadening. Roots occur scattered all over the rhizome except in *Bolbitis* and *Egenolfia* in which they are restricted to the ventral surface.

The ground tissue of the rhizome in all the genera is parenchymatous though scattered in it are present irregularly cylindrical sclerenchyma strands in *Bolbitis* (except *B. presliana*), *Egenolfia* (except *E. helferiana*) and

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the Dryopteroid ferns The vascular cylinder is solenostelic with a characteristic, broad, gutter shaped ventral vascular strand and a small, dorsal strand in *Bolbitis* and *Egenolfia* siphonostelic in the Dennstaedtioids and dictyostelic in the Dryopteroids Leaf traces originate in closely placed, spiral rows around the stelar cylinder in the Dryopteroid genera. They are restricted to the dorsal surface and are in two alternating, closely placed rows in the Dennstaedtioid ferns as well as in *Bolbitis* and *Egenolfia* (more than two rows occur in the adult stage in many species) The leaf traces are simple, gutter-shaped strands in the Dennstaedtioid genera. In *Bolbitis*, *Egenolfia* and the Dryopteroid ferns trace to each leaf consists of a large number of cylindrical bundles. Branching of rhizome is common among the Dennstaedtioids and the branch traces are usually simple cylindrical strands associated with the leaf traces and often unaccompanied by a branch gap. However in *Hypolepis* they are associated in a characteristic way with each leaf trace, the branches originating from the leaf trace after the latter has separated from the stelar cylinder of the rhizome and associated with small adaxially placed branch gaps In most species of *Bolbitis* and *Egenolfia* branch traces are associated with leaf traces the branch trace arising from the abaxial side of the leaf trace either along with one of the bundles of the leaf trace or separate from it. In *Bolbitis* and *Egenolfia* there is also an association of a root trace to each branch trace

The leaves are arranged in two alternate dorsal rows in *Bolbitis* *Egenolfia* and the Dennstaedtioid genera though in some species of the former two genera additional rows of leaves may be developed towards maturity In Dryopteroids on the other hand they are spirally arranged The leaf lamina is once pinnate in *Bolbitis* and *Egenolfia* while it is bipinnate to decompound in the other genera studied. Foliar vegetative buds occur superficially on the dorsal surface towards the apices of midribs of pinnae in most species of *Bolbitis* and *Egenolfia*, the terminal pinnae in some of the species being considerably prolonged and whip-like Foliar buds are absent in the Dennstaedtioid and Dryopteroid ferns.

The stipe in *Bolbitis* and *Egenolfia* possesses two, median dorsal grooves on the adaxial surface a single median adaxial groove occurs in the Dennstaedtioid and the Dryopteroid ferns Irregularly scattered sclerenchyma strand similar to those in the rhizome occur in the stipe towards its base in *B crispatula* *E asplenifolia* and the Dryopteroid genera Few of the outermost layers of the ground tissue of the stipe are progressively thick walled the thickening increasing in the outer layers. In *Arthropteris* and *Peranema* the epidermal and 2-3 layers of hypodermal cells are thin walled. Prominent aerenchyma strands interrupting the outer shell of thick walled cells occur on either side of the stipe, except in *Egenolfia* and *Dennstaedtia*. Vascular bundle of the stipe is single and gutter-shaped in the Dennstaedtioideae ; a number of slender cylindrical vascular strands arranged in a circle occur in *Bolbitis* *Egenolfia* and the Dryopteroid ferns. The venation is Go-

lopterod in *Bolbitis* but is free with a midrib bearing alternate lateral veins in the others. Multicellular uniseriate hairs are present on the lower surface of the lamina in all the genera. Mixed with them occur a few small paleae in *Bolbitis* and *Egenolfia*. The simplest juvenile leaf is obcuneate with a shallow terminal notch in *Bolbitis* and *Egenolfia* broad with deeply lobed outer margin in the Dennstaedtioid ferns and broadly obcuneate with an apical notch in the Dryopteroid ferns. The juvenile leaves bear club-shaped, multicellular hairs in *Bolbitis Egenolfia* and the Dennstaedtioid genera (also acicular hairs in the Dennstaedtioids) and unicellular papillate hairs in the Dryopteroid genera.

Extreme dimorphism of the fertile and sterile leaves characterize *Bolbitis* and *Egenolfia* the fertile leaves are similar to the sterile ones in the Dennstaedtioid and Dryopteroid genera. The fertile lamina of the former is highly reduced with acrostichoid distribution of sporangia and its venation pattern is simpler than that of the sterile lamina though of the same fundamental type. The sori are circular at the apex of the veins in the Dennstaedtioid and subapical on the veins in the Dryopteroid genera. Among the former they are protected by a cup-shaped indusium in *Dennstaedtia*, half cup-shaped indusium in *Microlepia* and by the reflexed margin of the leaf in *Hypolepis*. In the latter group they are protected by a globose indusium that ruptures irregularly at maturity the sori in *P ramosa* are stalked and the indusium splits along a vertical slit like opening. The sporangial stalk is two cells thick except at the capsule base where it becomes three (sometimes four in *Lithostegia*) cells thick by secondary development of a usually short row of cells from the basal wall cell abutting on the stalk. In the Dryopteroid ferns usually the 3rd row of cells extends nearly up to the base of the stalk. Short, club-shaped, multicellular foliar hairs occur mixed with the sporangia in all the genera and in some cases (*Acrophorus Diacalpe Lithostegia* and *Egenolfia sinuata*) occur attached to the sporangial stalks. The hairs in the Dryopteroid ferns are unicellular and more or less balloon-shaped.

The spores are monolete and enveloped by a characteristically folded perine in *Bolbitis Egenolfia* and the Dryopteroid genera. The perine is reticulate in *Egenolfia* but is generally psilate or granulate in the others. Spores of Dennstaedtioid ferns on the other hand are devoid of perine. They are trilete in *Dennstaedtia* and *Microlepia* but monolete in *Hypolepis*. The exine is usually psilate or granulate (verrucate in *D. elwesii* and spinate in *Hypolepis*)

The mature prothallus is cordate and massive in all genera. It is hairy in the Dryopteroids while in the others it is usually naked. In some species of *Bolbitis* and *Egenolfia* the prothallus has the tendency to elongate, often becoming strap-shaped. The mature prothallus bears sparse 2-4 cells long, curved, marginal hairs in *E. sinuata*. In the Dryopteroid genera the prothallus bears unicellular hairs very profusely the hairs being present from the

filamentous stage of development onwards. In all the genera studied the prothallus develops from a 4-6 cells long germ filament in which the anterior cells divide longitudinally to form a prothallial plate. An obconical apical meristematic cell is established soon afterwards and is replaced by a multicellular meristem when the apex of the thallus becomes cordate. In Dryopteroid ferns (except *Acrophorus*) the terminal cell of the germ filament usually ends in a hair and stops growth. Then the penultimate cell continues growth and the process may be repeated.

A phylogenetical evaluation of the morphological features of *Egenolfia* and *Bolbitis* is attempted. Trends of evolution among the different species of both the genera are traced and it is concluded that probably there are at least three lines of specialisation within the genus *Bolbitis*: one line is characterised by progressive condensation of the rhizome, the rhizome becoming short and thick as in *B. subcrenata* and *B. crenata* and finally developing more than two rows of leaves as in *B. deltigera*. In another set of species represented by *B. diversifolia*, *B. heteroclita* and *B. sinuata* the tendency is to have a long creeping rhizome which is slender and with two-ranked leaves in the early stages, but later on becomes vertically climbing, stout and bearing three or four rows of crowded leaves. A third line of specialisation is characterised by conspicuous reduction in the size of the plant and reduction of sclerenchyma strands of the rhizome as in *B. presliana*. Of these three lines of specialisation the different species of *Egenolfia* share the tendency for condensation of the rhizome and subsequent development of more than two rows of leaves as well as the tendency for reduction in size of the plant in some species.

The salient morphological features of *Egenolfia* and *Bolbitis* are compared with those of the Dryopteroid ferns, the Dennstaedtioid ferns, *Cyclosorus*, *Polystichum* and *Elaphoglossum*. An attempt is made to determine their probable relationship to these ferns. It is concluded that *Bolbitis* and *Egenolfia* are closely allied genera and together are related to *Elaphoglossum*, the three genera being probably derived from a common ancestor. With regard to *Cyclosorus*, *Polystichum*, the Dryopteroids and the Dennstaedtioids, detailed morphology of the sporophyte and gametophyte does not appear to support the suggestion of any close relationship to *Bolbitis* and *Egenolfia*.

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CONTENTS

A NOTE ON THE GENITALIA OF *SPHRACEPHALA HEARSEYANA* WESTW (DIOPSIDAE DIPTERA)*

J L. NAYAR AND SANTOSH K. TANDON
School of Entomology St John's College Agra.

INTRODUCTION

This paper deals with the morphology of genitalia of *Sphracephala hearseyana* Westw and is in continuation of the earlier two papers (Nayar and Santosh K. Tandon, 1962 A and B) on the morphology of head capsule and wing venation. Singh, Nayar and Tandon (1962) have studied the thoracic morphology of this fly Account on other systems will follow in subsequent papers

MATERIAL AND METHOD

The Diopsid flies for this work collected from the leaves of *Phoenix* sp (Palm) and *Ageratum* sp. during September and middle of October mainly from the Botanical Garden St. John's College Agra, were partially decolourised by chlorine fumes, washed through the grades of alcohol and finally dissected in canada balsam for detailed study Diagrams were drawn with the help of camera lucida The terminology followed here is after Van Emden and Hennig (1956)

THE MALE GENITALIA

The morphologists refer the appendages of the post-abdomen as genitalia, hypopygia, genital apparatus 1 armature genitale armature copulatrix geschlechtsanhange terminalia pygidia etc. as reported by Metcalf (1921) In *Sphracephala hearseyana* Westw the hypopygium (Figs. 1 2,3 and 4) has been formed by the highly modified ninth abdominal segment. Normally the terminal end of the abdomen remains curved downwards so as to make the genitalia lie ventral to the seventh abdominal segment. The eighth segment is greatly reduced and does not contribute towards the formation of the genitalia. The absence of spiracles on the ninth segment render the differentiation of tergal and sternal portions impossible. Nevertheless ninth tergum or epandrium (T_9) seems to be represented by a highly sclerotised horse-shoe-shaped piece bearing styles (SURS) and claws (CLW) at the inner margin of the terminal end of the horse-shoe-arms. Lundbeck (1916) and Nayar (1961) are of the opinion that the style bearing segment represents the ninth segment in Diptera Metcalf (1921) however considers it to be a urite a term denoting the segment with indistinguishable tergum and thus assumes that the whole structure behind the eighth tergum alongwith the styles and claws represents the tenth tergum in Syrphidae. Berlese (1909) believed that the first apparent segment of the abdomen in Deptera is the actual third so the

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terminal segment may well be called as the eleventh segment but Ferris (1950) on the other hand holds that the first apparent abdominal segment is in reality the second in Diptera a view which has been supported by Singh, Nayar and Tandon in *Sphracephala hearseyana* Westw (1962) In view of Ferris s interpretations it is evident that the hypopygium really represents the ninth abdominal segment.

The ninth tergum or epandrium (T_9) posteriorly bears a pair of appendages called the surstyli (SURS) which have been differently called by various workers as mesostyli appendage I claws and forceps inferiores. Each surstylus (SURS) is a bent lobe-like structure with strongly convex inner margin and concave outer margin. They act as claspers and are beset with fine bristles along the concave margin Lateral to the surstylus (SURS) at the base there is present a small claw (CLW) which appears to be tactile in function. Along the interior surface the ninth tergum bears a pair of cerci representing the tenth segment. Each cirrus (CRS) is a spindle-shaped structure covered all over by fine prominent bristles. Cirri lie parallel to one another along their inner margin.

The ninth sternite or hypandrium is modified into a complex of the penis sheath (PS) inferior lobes (IL) superior lobes (SL) the sustentacular apodeme or phallapodeme (SUSA) and the chitinous box (CB) The penis sheath is cylindrical, heavily chitinised at the caudal end but relatively weakly sclerotised at the cephalic end It is articulated to the posterior border of the sternum by its basal rim, the cephalic half of which is semi-circular but caudal half is pointed. The lingula (LGA) or the heavily sclerotised caudal part of the penis-sheath bears apically the inferior and the superior lobes (IL & SL) superior lobes being disto-caudal in position to the former The aedeagus (AED) or the penis proper also known as phallus or theca or appendage III or the intromittent organ is an unpaired median structure representing a part of the ninth sternite or hypandrium (S_9) Distally the penis is modified into a heavily chitinised hood like structure called the chitinous box (CB) by Berlese (1909) It carries the ejaculatory orifice (EJAO) at its apex. The sustentacular apodeme or phallapodeme (SUSA) has been referred to as the double apodeme by Wesche (1906) and he considers it to be a paired organ in many families of Diptera. In *Sphracephala hearseyana* Westw however it is a simple rod like structure extending to the base of the chitinous box (CB) It possibly works as a supporting organ during copulation and apically bears sharp fine ridges or keels (K)

The Female Genitalia

The eighth and the ninth segments of the female abdomen constitute the so called telescopic 'ovipositor' Snodgrass (1935) calls it the 'substitutional ovipositor' in Diptera The eighth tergum (Figs. 5 and 6 T_8) is sclerotised only laterally and bears ventrally two distinct plates the gonopods (GPD) of Ferris (1950) enclosing centrally the vulva (VLV) These plates are conne-

cted at their bases by a transverse sclerotised bar probably representing the subgenital plate of Snodgrass (1935) The ninth tergum is membranous bearing postero-laterally a pair of lobes which are covered with bristles. The ninth sternum (S_9) is also membranous bulging into a thin pad like hemispherical lobe meant probably for securing anchorage on the leaf at the time of oviposition

Summary

The hypopygium are the modified ninth abdominal segment, and comprise the surstyli, claws cerci, penis sheath inferior lobes superior lobes the aedeagus, the ejaculatory apodeme the sustentacular apodeme or phallapodeme and the chitinous box. The female genitalia are formed by the eighth and ninth segments, the female genital opening lying between the gonopods of the eighth sternum.

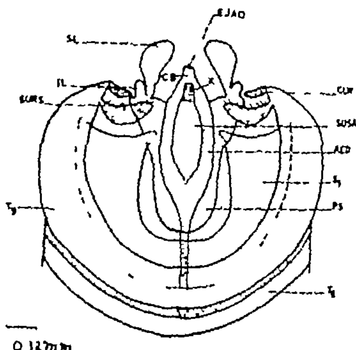
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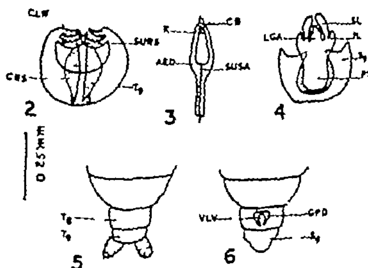
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AED—Aedeagus CB—Chitinous box; CLW—Claw; CRS—Cercus, EJAO—Ejaculatory opening; GPO—Gonopod IL—Inferior lobe, K—Keel; LGA—Lingula PS—Post. arch, S_9—Sternum, SL—Superior lobe; SURS—Surstylus; SUSA—Sustentacular apodeme T_8—Tergum, T_9—Tergum; VLV—Valva.

Fig 1 Male genitalia (semi-diagrammatic).
Fig 2 Dorsal view of the terminalia.
Fig 3. Aedeagus and associated structures.
Fig 4 Ventral view of the terminalia with the aedeagus removed.
Fig 5 Dorsal view of the female terminalia.
Fig 6. Ventral view of the female terminalia.

MORPHOLOGY AND STERILITY OF THE POLLEN GRAINS OF SUB-TROPICAL PLUMS

S. N. Singh
Horticultural Research Institute Saharanpur

It has long been known that in many plant species the apparently normal flowers produce some amount of defective pollen and that it is unusual to find pollen which is 100 per cent morphologically perfect.[14] The varying proportions of defective pollen have a direct bearing on the fruitfulness of a variety

Dorsey[3] and Griggs[8] reported a low pollen fertility in Japanese plums (*Prunus salicina* Lindl.) while Flory and Tomes[5] studied the appearance of plum pollen and stated that pollen sterility is a character which may be used as an indication of species relationships. Randhawa and Nair[13] reported total pollen sterility in the variety Alucha Yellow under Delhi conditions and suggested the examination of its pollen in other localities. They also found a wide range of size variation from 86·4 to 105·8 microns in the pollen of five sub-tropical plum varieties.

The observations of above workers prompted the author to undertake the study of the morphology and sterility of the pollen of the plum varieties being grown in the sub-tropical conditions at Saharanpur with a view to elucidate the information about their pollen condition It was also thought desirable to locate the total and partial-male sterile plum varieties for suggesting their interplantation with suitable pollenizers

Materials and Methods

Healthy and comparable trees of thirteen varieties of country plums (*Prunus salicina* Lindl.) and seven imported plums growing at the Horticultural Research Station, Saharanpur were selected for the study The pollen grains from freshly dehisced anthers were collected after anthesis and mounted on slides in methyl-green glycerine jelly according to the method devised by Wodehouse.[11] For describing the pollen grains, Erdtman's system of classification[4] was followed. The size of the normal and perfect grains was measured in the polar view along the longest spore axis with the help of a Zeiss Ocular 10 X micrometer whereas in the case of sterile and imperfect grains, only the maximum length was recorded

The pollen sterility of each variety was examined in the middle and end of the blooming season by the aceto-carmine staining reaction Under the microscope, the fertile and normal grains looked plump and deeply stained while the sterile and imperfect ones appeared shrivelled, empty and unstained.

The number of these grains in 20 random fields on four slides was counted separately and the percentage of sterile pollen was calculated. While examining the slides care was taken to include fields lying at the peripheral regions of the cover-slips, since the abortive pollen grains often tend to accumulate near the periphery

OBSERVATIONS

A. *Pollen Morphology*

(a) *Symmetry Aperture and Shape*—The mature and normal pollen grains of all the varieties were similar in morphology In the natural dry state, they were ellipsoidal while in methyl-green glycerine jelly mounts they readily took moisture expanded and became oblately flattened and angular in outline (Fig 1)

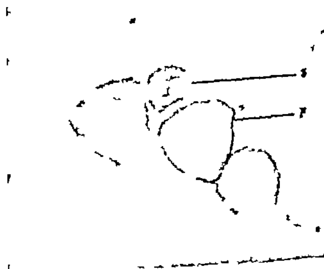


Fig 1 Pollen grains of plum Jamuni in methyl-green glycerine jelly mount, X356. S=Sterile and F=Fertile grains.

The pollen grains were isopolar fusiform, radiosymmetric and 3-aperturate The position of apertures was equitorial with angulaperturate amb-type. The grains were 3-colporate. The exine was fairly thick and finely striate. The shape of the pollen grain was sub-prolate and they were observed to shed at the 2-celled stage.

(b) *Size*—Normal and abortive both types of pollen grains were found to occur in all the varieties in varying proportions. The average diameters and size ranges of the pollen grains of the plum varieties are summarized in Table 1

A perusal of Table 1 shows that the average diameter of all the plum varieties lay between 33 33 to 38 94 microns, indicating negligible variation. The range in size varied from 29·7 to 46·2 microns. Of all the varieties, the average lengths of the pollen grains of Howe and Excelsior were the

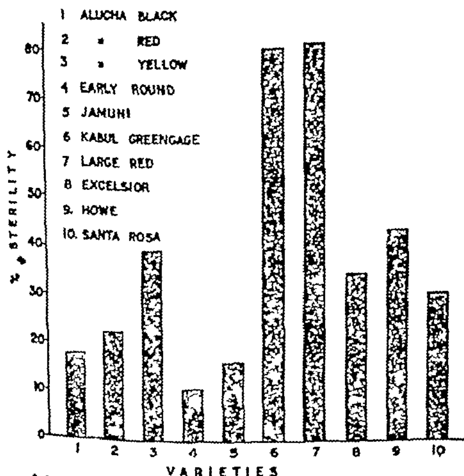
largest being 38·94 and 38·28 microns respectively while the sizes of Large Red and Early Large Red were the smallest being 33 33 and 33 99 microns, respectively The pollen-sizes further indicated that the grains of all the twenty varieties belonged to the medium size spores class Varieties Dwarf Early Yellow Large Yellow and Late Yellow were found to be devoid of normal and plump pollen grains.

The average maximum lengths of the imperfect and sterile pollen grains ranged from 19·47 to 21 78 microns.

B. *Pollen sterility*

The percentages of imperfect and empty pollen grains recorded in the peak and end bloom of 20 varieties of plums are given in Table 2 and partially depicted in Fig 2.

FIG.2— POLLEN STERILITY PERCENTAGE OF PLUM VARIETIES



It is evident from Table 2 and Fig 2 that the country plums—Dwarf Early Yellow Large Yellow and Late Yellow varieties produced only abortive

pollen and showed total male sterility Among the remaining varieties, Large Red and Kabul Greengage showed the highest average pollen sterility of 81·83 and 80·56 per cent respectively which indicates that they produce negligible amount of fertile pollen. *Varieties* Howe, Alucha Yellow and Excelsior exhibited high average pollen sterility of 43 75 39·59 and 35 09 per cent respectively The lowest average pollen sterility of 11 03 per cent was shown by Early Round, which was followed by Alpha (13 96 per cent) and then by Kelsey's Japan (14 07 per cent) varieties These figures evidently indicate the high pollen-fertility status of these varieties.

Discussion

The present investigations have shown that the pollen grains of the plum varieties under study are identical in morphology The average size of the normal pollen measured along the longest spore axis ranged from 33 53 to 38·94 microns showing slight variation These observations are in conformity with those of Wodehouse[14] who reported that the pollen sizes of the members of rosaceae family range from 25 to 52 microns. These results do not however tally with those of Goff[9] who observed that *Prunus* hybrid trees produced pollen which were 0·388 mm. long and 0·22 mm. wide. Randhawa and Nair[13] have recently measured the pollen lengths of five varieties of sub-tropical plums which range from 86 4 to 105 8 microns While Goff's[9] observations appear to be an approximate estimation of the pollen size, the results reported by Randhawa and Nair[13] need to be confirmed further by undertaking size measurements of a larger number of sub-tropical plum varieties in Delhi conditions

In the course of study of pollen sterility Dorsey[7] found pollen abortion to be common in plums and observed 74 to 83 per cent pollen viability in Japanese plums Griggs[8] held the same view and stated that the Japanese plums produce scanty amount of pollen with low viability In the *Domestica* group of plums Crane and Lawrence[4] found one variety Golden Esperen to be totally male sterile and useless as a pollenizer Among the sub-tropical plums Randhawa and Nair[13] found Alucha Yellow variety to be devoid of normal pollen In the present tests, however out of 20 varieties examined Dwarf Early Yellow Large Yellow and Late Yellow have been found totally male sterile while Alucha Yellow variety has shown partial pollen sterility of 39 11 to 43 08 per cent. These varieties are totally unsuitable pollenizers for optimum fruiting and should never be planted as sole. Of the remaining plum varieties Kabul Greengage and Large Red exhibit a very high (80 56 to 81 83) and Excelsior and Howe a high (35 09 to 43 75) percentage of defective pollen indicating partial male sterility and thus necessitating the inter-planting of pollenizers for normal fruit setting Griggs[8] has suggested that the varieties having high percentage of abortive pollen should be interplanted with a pollenizing variety in every sixth row The high percentage of pollen sterility of varieties Alucha Black Alucha Red that

Excelsior and Howe obtained in the present studies resemble closely with those reported by Randhawa and Nair[13] and slight variation in some cases might be attributed to climatological factors

While investigating the causes of total male-sterility in Washington navel orange, Osawa[11] Webber[13] and Frost[7] reported it to be due to the degeneration of the primary sporogenous cells or the pollen-mother cells Dorsey[5] has found that disintegration in the plums usually occur after the liberation of the tetrad from the pollen-mother cell. On the basis of these observations, it may be concluded that in the plum varieties with usually low percentage of defective pollen the degeneration occurs in the process of the development of the male gametophyte. Sterility of the pollen has also been recognized as a condition frequently associated with hybridity and generally the wider the crossing the greater is the degree of sterility encountered[3] Flory[6] held the same view in plums and stated that the general level of pollen sterility depends on the degree of hybridity The high percentage of pollen sterility of the plum variety Excelsior is due to the fact that it a hybrid of the cross *Prunus triflora* Roxb X *P munsoniana* W & H. This view find its further support from the observations made by Randhawa and Nair The other plum varieties, viz Large Red Kabul Greengage, Alucha Yellow and Howe with high percentage of sterile pollen may perhaps have originated a chance seedlings from natural crosses It will be interesting to study the meiotic chromosomal behaviour of these varieties which may elucidate the possible causes of total and partial male sterility

SUMMARY

1 The perfect and mature pollen grains of the sub-tropical plum varieties were 3-colporate, sub-prolate and similar in morphology while the sterile grains were devoid of protoplasmic contents.

2. The average diameters of the normal pollen ranged from 33 33 to 38·94 microns and they belonged to the medium size spores class. The average lengths of defective pollen varied from 19 47 to 21 78 microns

3 The plum varieties—Dwarf Early Yellow Large Yellow and Late Yellow produced 100 per cent defective pollen while varieties Large Red, Kabul Greengage, Howe Alucha Yellow and Excelsior exhibited high grades of pollen sterility indicating partial male-sterility These varieties need to be interplanted with suitable pollenizers for optimum fruiting

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TABLE 1

Size of pollen grains of plum varieties

S No	Varieties	Normal pollen grains		Av. length of sterile pollen grains in μ
		Range in size in μ	Av. diam in μ	
	A. *Indigenous Plums*			
1	Alubokhara Large	29 7—39 6	35.97	20 13
2	Alucha Black	33 3—39 6	36 63	21 12
3	Alucha Red	33 3—46 2	35 97	21 12
4	Alucha Yellow	29 7—39 6	34 32	20 46
5	Dwarf Early Yellow	Total Male sterile	...	21 12
6	Early Large Red	29 7—39 6	33 99	20 13
7	Early Round	29 7—39 6	34.32	19 47
8	Jamuni	29 7—42 9	36 96	21 78
9	Kabul Greengage	33 3—39 6	35 31	18 81
10	Ladakh	29 7—39 6	37 29	21 12
11	Large Red	29 7—36 3	33 33	20 46
12	Large Yellow	Total Male Sterile	...	19 80
13	Late Yellow	Total Male Sterile	...	20 46
	B. *Imported Plums*			
14	Alpha	29 7—39 6	34 32	20 79
15	Excelsior	33 3—46 2	38.28	20 79
16	French Red	33 3—39 6	35 64	21 78
17	Howe	33 3—46 2	38 94	21 78
18	Kelsey' Japan	29 7—36 3	34 32	21 45
19	Santa Rosa	33 3—36.3	34 98	21 78
20	*Prunus* [illegible]	33 3—39 6	34 65	21 12

TABLE 2

Percentage of pollen sterility in plum varieties determined by aceto-carmine staining reaction

S No	Varieties	Per cent pollen sterility* In mid-bloom	In end-bloom	Average	Remarks
	A. *Indigenous Plums*				
1	Al bokhara Large	28 09	32 06	30 07	
2	Alucha Blr k	14 79	20 61	17 71	
3	Alucha Red	20 69	24 63	22 06	
4	Alucha Yellow	36 11	43 08	39 59	
5	Dwarf Early Yellow	100.00	100 00	100 00	Total male sterile
6	Early Large Red	14 69	16 45	15 57	
7	Early Round	10 04	12 06	11 05	
8	Jamuni	13 43	19 22	16 3.	
9	Kabul Greengage	79 66	81 47	80.56	
10	Laddak	22 75	24 08	23 41	
11	Large Red	79 83	83 81	81 83	
12	Large Yellow	100 00	100 00	100 00	Total male sterile
13	Late Y llow	100 00	100 00	100 00	Total male sterile
	B. *Imported Plums*				
14	Alpha	12 84	15 09	13 96	
15	Excelsior	34 35	35 83	35 09	
16	French Red	24 44	32 14	28 29	
17	Howe	41 10	46 41	43 75	
18	Kelsey J pan	12 91	15.21	14 07	
19	Santa Rosa	28 10	33.56	30 83	
20	*Prunus simoni*	15 15	1 29	18 22	

Based on more than 500 pollen grains.

STUDY ON EXTENSIVE AIR SHOWERS OF COSMIC RAYS IN THE ENERGY INTERVAL 10^{11} E VOLTS TO 10^{14} E VOLTS AT NAINI TAL

PART I

B. N. SRIVASTAVA

Assistant Professor of Physics D. S. B. Govt College Naini Tal

GENERAL INTRODUCTION

When Cosmic rays enter the atmosphere they produce nuclear interactions with the nucleus of the atmospheric gases. In these high energy collisions the Primary Cosmic ray particle loses a small fraction of its energy in multiple π-meson production. At large energies K-mesons, hyperons and nucleon-antinucleon pairs might also be produced. The Primary particle continues to produce successive nuclear reactions till its energy degradation is such that further meson producing interactions become impossible. The secondary mesons and antinucleons produce further nuclear reactions, decay or get annihilated. A nuclear cascade process thus develops around every high energy primary that enters the atmosphere. In case of low energy primaries of energy ≈ 10^{13} e. volts this cascade is quickly absorbed into the atmosphere before striking the ground (except for a few μ-mesons that posses large penetrating power and relatively larger life time).

The situation is different at primary energy ≫ 10 e. volts. The nuclear cascade continues to develop upto larger depths into the atmosphere and showers of secondary particles can be observed at mountain altitudes as well as sea level corresponding to the primaries of energy ≫ 10 e. volts.

In meson producing collisions at least one third of the mesons are π^0-mesons. These mesons play a very important part in the production of the electronic cascade. π^0 mesons decay almost instantaneously into two photons. Through pair production, annihilation and bremsstrahlung the electron cascade develops so rapidly that for all practical purposes the electron cascade dominates over all other constituent particles of an air-shower. Hence although the shower is initiated and constantly fed by nucleonic component, its main part consists of electrons and photons. It is because of this fact that the old results of electron cascade theory still remain valid for calculations of density distribution of charged particles. However there are some other important features which clearly prove that shower generating particles are different from electrons or Photons and are nucleonic in nature. Some of these structural features which throw light on the detailed behaviour of showers have been studied in this Laboratory.

Introduction—The object of the work undertaken since 1958 was to study (a) the anisotropy if any in the direction of arrival of the primary particles in solar as well as sidereal time, (b) the nature of the energy spectrum of these particles (c) Absorption coefficient of the showers through the study of Pressure coefficient and its correlation with the energy exponent of these showers. Present paper deals with the technique of study of Extensive Air Showers with G. M. Counter-arrays. Some results obtained regarding the flux of different energy showers the energy spectrum and the pressure correlations of showers in solar time are presented in this article.

Efficiency of G. M. Counters as particle density selectors—The probability that a G. M. Counter records a density Δ of particles depends upon the area of the counter

$$P = (1 - e^{-\Delta a})$$ where a is the area of the counter and Δ the density of particles.

If n counters of the same area are connected in coincidence then the probability for recording n—fold coincidence becomes

$$P_n (\Delta\ a) = (1 - e^{-\Delta a}) \qquad \text{(Ref No 4)}$$

The coincidence rate which is proportional to the above probability and the density distribution function will be

$$R_n = K (1 - e^{-\Delta a})^n \times F(\Delta)$$ where K is some constant depending upon the source strength and the efficiency of the instrument. In case, the source strength is constant and the counter efficiencies remain the same K becomes a constant.

If one uses triple coincidence trays of the same effective area the counting rates will be

$$R_3 = K(1 - e^{-\Delta a})^3 F(\Delta)$$

In case of showers the density distribution function is very well known

$$\gamma(\Delta) d\gamma = K\ \Delta^{-\gamma} d\Delta$$

Hence the triple coincidence rate will be

$$R_3 = K(1 - e^{-\Delta a})^3 \Delta^{-\gamma-1}$$

It can be shown that about 80% of the triple coincidence rates are recorded for which $\bar{\Delta} \simeq \frac{1}{a}$ which means that for a given effective area of counter trays, the trays will record showers of a certain average density preferentially

Lateral distribution function of shower particles and estimation of energy—A detailed theory of lateral distribution function has been given by Nishimura and Kamata (1950 51 52)[1 2 3] A simple approximate relation has been used here for our analysis. The distribution of shower particles from the core out-wards is given by $\Delta(\ ) = \frac{N f(r)}{R_o^2}$ where $f(r)$ is a function of distance from the core and the age of the shower N is the total number of charged particles and r is the cascade length in meters An approximate relation $\Delta(\ ) = \frac{N}{2\pi R_o} \frac{e^{-\gamma/R_o}}{\gamma}$ also gives the density distribution fairly accurately (Galbraith—1958)[4]

The total number of particles associated with a shower striking an array of triple coincidence counters separated by a distance and placed at the corners of an equilateral triangle can be estimated. But the total number of charged particles at the shower maximum are related with the Primary energy as follows —$E \approx 10^{10} \times N$ e. volts (Galbraith—1958)[4] Hence the energy of the Primary producing the shower can be estimated

Energy estimation from the Lateral distribution function—If a shower strikes the array of triple coincidence counters, its core might be either lying outside the triangular lattice or inside

The distance of the core from all the three counters will be smallest when its core falls at the centre of the triangular array The average density recorded by the trays will therefore be given by

$$\bar{\Delta} > \frac{1}{a} > \frac{N}{2\pi R_o} \frac{e^{-\frac{d}{\sqrt{3} R_o}}}{\frac{d}{\sqrt{3}}}$$

$$\text{or} \quad N = \frac{2\pi R_o d}{a\sqrt{3}} \; e^{\frac{d}{\sqrt{3} R_o}}$$

The energy of the showers will be

$$E > 10^{10} N > \frac{2\pi R_o d}{a\sqrt{3}} \; e^{\frac{d}{\sqrt{3} R_o}} \; 10^{10} \text{ e. volts}$$

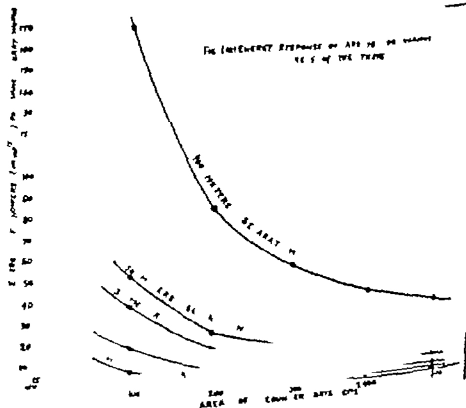
The energy of showers recorded by arrays with various distances of separation of counter trays of different areas has been theoretically calculated. The table I below gives the details. Fig 1 (a) shows how the energy recorded by an array changes with distance of separation and with the area of trays.

For the selection of a particular geometry one is left with not much of choice as the space available in the Laboratory has to be utilised for erection of arrays. We selected counter trays of 500 sq cm. area. The distance of separation was taken to be 8 meters, 20 meters, 30 meters and 45 meters for

four groups of energy selection The counters were placed on the roof of the upper Physics Lab of D.S.B. Govt. College with a few gm cm² of wooden and metallic covering The absorption due to this factor was accounted for in the energy calculation.

TABLE 1

Sl No	Area of Trays	Energy of showers in 10^{14} e volts for various distances of separation					
		10 m	20m	30 m	40 m	50 m	100 m.
1	100 sq cm	6 94	15 34	25 43	37 47	51 75	170 07
2	200 sq cm	3 47	7 67	12 72	18 74	25 72	85 91
3	300 sq cm	2 31	5 11	8 48	12 49	17.25	56 09
4	400 sq cm	1 74	3 84	6 36	9 37	12 91	42 32
5	500 sq cm	1 39	3 07	5 07	7 49	10.35	34 01



Experimental arrangements—Initially two triple coincidence arrays with a separation of 8 metres and 20 metres were used to select 5.4×10^{14} and 3.07×10^{15} e. volt showers The area of the trays was 470 sq cm. each The counter trays were placed under the roof of the upper Physics Laboratory of the Physics Department There was some absorption of shower particles due to some matter above the trays This caused an upward shift in the energy of showers recorded by arrays and rates were naturally reduced This factor was later investigated by taking the counters on the roof with minimum covering matter and using the same geometry

In order to investigate 5×10^{15} e. volt and 10^{1} e volt showers as well a multiple array of four trays was used. The diagram below gives the layout of the counter trays.

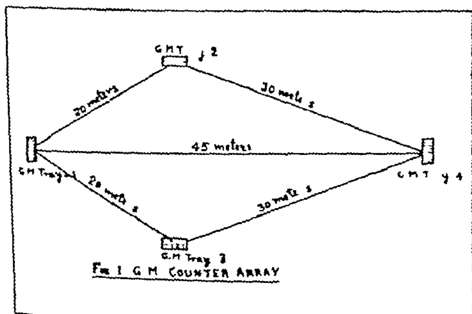


FIG 1 G M COUNTER ARRAY

The counter trays (1 2,3) (2,3 4) and (1,2,3 4) were arranged in coincidence. These coincidences were recorded on a H B. pen recorder on 24 hour time scale. The three group of coincidences corresponded to three different energies of showers Thus showers of energies 3.07×10^{15} 5.07×10^{15} and 8.9×10^{15} were simultaneously recorded by this array

Electronic Circuits—G M. Counters manufactured by T I F R Bombay were used in the present work. With a suitable electronic quenching circuit (with 6-AK 5 tube in Neher Harper Circuit) the performance of the counters was satisfactory

The working voltages of counters were checked daily to make sure that counter conditions remain the same All supplies L. T as well as H. T were electronically stabilised so that the voltage fluctuations were extremely

small. The local A. C. Mains supply was also stabilised by using a constant voltage transformer

The voltage pulse after the 6-AK 5 quenching cathode follower circuit was brought to a central recording point through RG 11/U cables. These pulses were not of the same size and were attenuated differently. An amplifier and invertor stage was therefore used with each tray and all the pulses were brought to the same size and form before feeding them to coincidence circuits. The details of one such recording channel are given in Fig. 2 below and the circuit layout for the complete array is shown in Fig 3

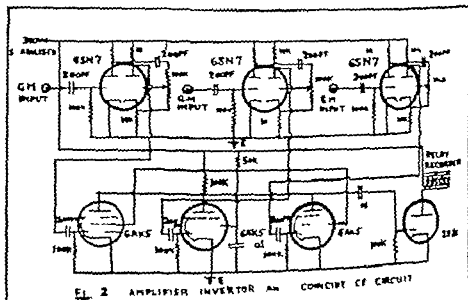


Fig. 2 AMPLIFIER INVERTOR AN COINCIDT CF CIRCUIT

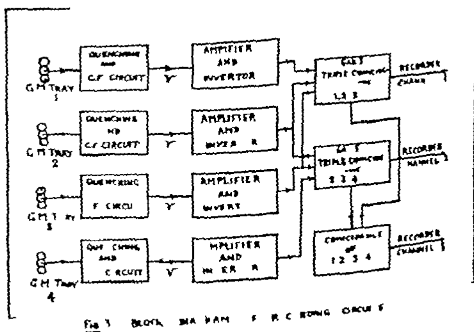


Fig 3 BLOCK DIA RAM F R C RDING CIRCU F

FLUX OF HIGH ENERGY PRIMARIES

Showers in the energy groups 5 44×10¹⁴ e. v 3 07 ×10¹⁵ e. v 5.07 ×10¹⁵ e. v and .89×10¹⁶ e v have been recorded by separate groups of counter trays in the combined array From the shower rates the integral flux of particles producing these showers has been determined. Table 2 below gives the details of the analysis.

TABLE 2

Sl. No	Counter Separation	Effective area of the array in m²	Energy recorded in volts	Rates of showers/hour	Flux of primaries in No. of particles cm⁻² sec⁻¹ Sterad⁻¹
1	8 meters	67 4	15 44×10¹⁴	15 74± 140	10 125± 09 × 10⁻⁹
2	20 meters	316 2	3 07×10¹⁵	16 28± 145	2 01± 018 × 10⁻⁹
3.	30 meters	803 8	5 07×10¹⁵	15 35± 143	8 4± 08 × 10⁻¹⁰
4	45 meters	1503 8	89×10¹⁶	7 05± 095	1 93± 026 × 10⁻¹⁰

The effective area within which the shower axes are to fall so that they might be recorded by a particular group of counters in the array has been taken to be the area enclosed by a circle drawn with three G M. counters on its circumference. The solid angle from which the showers arrive at the array has been estimated from the pressure coefficient of these showers at Naini Tal. $\omega = (8.5/B+2.6)$ sterad. (Galbraith)[8] whe e ω is the solid angle in steradians and B is the pressure coefficient in per cent per cm. of Hg Pressure coefficient of 5×10¹⁵ e. v showers (Srivastava et al. 1961) has been used for calculations of solid angle. The flux values have been plotted cor es-ponding to the estimated energy of showers in Fig 4 The integral frequency Energy Spectrum of Primaries producing these showers may have the form $\eta(>E) = KE^{-\gamma}$ $\eta(\geqslant E)$ is the number of showers of energy $\geqslant$E K is a constant and γ is the exponent in the energy spectrum There seems to be a change in the value of γ in the energy region 10¹⁵ to 10¹ e. volts Its value at 5·07×10¹⁵ e volts is 1 71 and at 8.9×10¹⁵ e volts it changes to 2 1

Variations of the Extensive Air Shower rates in Solar time and their pressure correlations—Hilberry (1941) Kraybil (1949) Hodson (1955)[9] and Cocconi and Toongorgi (1949)[9] studied the altitude effect of these showers with a selection system that recorded showers rates particularly towards lower energy group at various altitudes. It was shown that below a height of about 8.2 Km. in the

atmosphere the showers show an exponential absorption. The shower rates as a function of atmospheric thickness t could be expressed as $R(E, t) \propto e^{-t/\Lambda}$, where $\frac{1}{\Lambda}$ (140 gms. $Cm^{-2})^{-1}$ and is called the absorption or attenuation coefficient of showers.

This very important property of cascade processes that gives an information about the nature of absorption of the showers in the atmosphere, could also be studied inside the laboratory space at a constant altitude for a wide range of shower energies through pressure correlation of shower rates. The atmospheric pressure over a place is known to have diurnal and semi-diurnal variations in solar time. If an array of G M Counters or scintillation counters is situated at a constant altitude, these small but regular pressure fluctuations that take place daily will cause a shift in the effective altitude of the recording system. An increase in the atmospheric pressure will amount to a downward shift in altitude with a corresponding decrease in the shower rates and a decrease in the atmospheric pressure will mean an upward shift in altitude with an increase in the shower rates.

The shower rates in this way could be expressed as $R(E, t) = Ke^{-Bp}$ where p is the atmospheric pressure in Cm. of Hg B the pressure coefficient or barometric coefficient and K is a constant for a particular array of counters. The barometric coefficient is written as

$$B = -\frac{1}{R}\frac{dR}{dp} \text{ or } -\frac{d \log R}{dp}$$

If there are no other sources of fluctuations in the shower rates then the barometric coefficients will be practically the same as the absorption coefficient defined earlier (A small temperature coefficient could be neglected in comparison to a much larger pressure coefficient.) A study of daily shower rates in relation to changes in the barometric pressure provides a method on a laboratory scale for the study of the absorption coefficient of different groups of showers for various energies of primaries. Some systematic measurements of barometric coefficients has been done by Daudin (1953) and Cranshaw (1957). Cranshaw has shown that the barometric coefficients change from 10% to 16% per cm of Hg in the energy interval 10^{14} to 10^{17} e volts. The showers ch large pressure coefficients have shown larger solar diurnal and semi-diurnal amplitudes as well. If the cosmic ray primaries of energy $\geq 10^{14}$ e volts have the same relative abundances as the primaries below the energy of 10^{13} e. volts then the occurance of a large diurnal amplitude and barometric coefficient cannot be properly explained.

We have attempted to study two different kinds of showers in relation to the barometric fluctuation and have determined their diurnal and semi-diurnal solar amplitudes as well. The first group of showers allows all the secondary electrons and μ-mesons to trigger the G M Counters used in the

array but the second group of showers have been recorded with an absorber (> 30 gm cm²) over G M Counter trays that removes low energy electrons and only energetic electrons or μ mesons are able to enter the G M Counters. Since the effective area of trays and their mutual separation is not altered in this selection system the two groups of showers will select showers of different energies and different kinds of secondary particles.

After studying the situation created due to a local absorber showers of 3.07×10^{13} e. volts energy group were studied with an absorber (>30 gm.cm^{-2}) and without any appreciable absorber In these two groups 44000 and 52000 showers respectively were collected in solar time from July 1960 upto March 1962. Daily pressure records were maintained for the local pressure with the help of a barograph supplied to us on loan by the Department of Meteorology Govt. of India. The shower rates as well as the pressure data have been analysed for diurnal and semi-diurnal variations in solar time and the degree of correlation with pressure has been determined for both the shower groups

Analysis of Solar variations of shower rates and pressures—Shower rates have been grouped in bi-hourly intervals in solar time for monthly periods as well as for the entire duration of observation The data has been harmonically analysed in solar time. It is highly unlikely that sun emits primary particles of extensive air shower energies (i. e. $\geqslant 10^{14}$ e volts) Whatever fluctuation one can foresee in the rates of such showers will be due to local modulation in rates produced in the atmosphere mainly due to atmospheric pressure variations

For harmonic analysis, various Fourier components could be fitted into the data of shower rates as follows —

$$R(t) = A_0 + (A_1 \cos t + B_1 \sin t) + (A_2 \cos 2t + B_2 \sin 2t) + \ldots \quad \text{etc.}$$

For $n \geqslant 1$ various components could be written in general as

$$(A_n \operatorname{Cos} nt + B_n \sin n\, t) = C_n \sin (nt + \epsilon n)$$

$$\text{where } A = C_n \sin \epsilon n \quad \text{and} \quad \frac{A_n}{B_n} = \tan \epsilon n \quad \sqrt{A^2 + B_n^2} = C_n$$

$$B_n = C_n \operatorname{Cos} \epsilon n$$

The time of maxima

$$t\,(\text{max.}) = \frac{1}{n}\,(\pi/_2 - \epsilon n)$$

Following Chapman and Bartels[1] different harmonic amplitudes and phases of maxima could be determined by a sum and difference method from a set of equidistant points on a harmonically varying curve We have used this method for finding the 1st and 2nd harmonic amplitudes and phases of pressure oscillations as well for the analysis of shower rates in the two groups.

Pressure oscillations over Naini Tal—Pressure oscillations seem to be quite regular and periodic in nature. Some very sharp changes were also recorded

during a stormy weather but these local fluctuations are averaged out in a data of a long period. The Harmonic dials of 1st and 2nd pressure harmonics for different months in the year are shown in Fig 4. There are small fluctuations in the amplitudes and also in the phases of harmonic vectors. The overall analysis for 13 months data gives a 1st harmonic amplitude of fluctuations (maxima to minima) equal to 0 63 gm. cm^{-2} and a 2nd harmonic amplitude of fluctuations equal to 2·80 gm. cm^{-2} at 17·2 and (0·64 and 12·64) hours respectively It is clear from these values that the diurnal amplitude is not so prominent as the semi-diurnal amplitude.

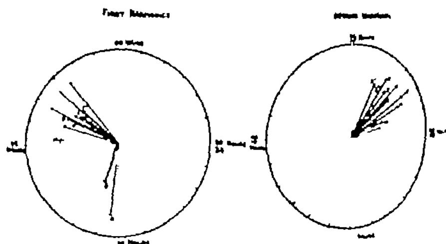


Fig (4) HARMONIC DIAL OF DIURNAL AND SEMIDIURNAL PRESSURE AMPLITUDES FROM ...

Analysis of Shower Rates in Solar Time

Showers have been grouped in bi-hourly intervals for monthly periods 1st and 2nd harmonic amplitudes and time of the occurrence of their corresponding maxima has been determined for each month and also for the entire period under the two groups. The following tables (Nos. 3 and 4) give the details of the analysis

If the combined data of the two groups is compared (last row of the tables 1 and 2) it becomes immediately clear that the phases of maxima in both the groups are approximately the same but the amplitudes of 1st as well as 2nd Harmonics are much different. In the first group where no absorbing material is placed over the trays, the amplitudes are much larger than 2σ level of the error whereas the amplitude of showers in the second group are submerged below the 2σ level.

This observation is quite significant as it shows that the penetrating showers striking the array centrally are less sensitive to solar variations

TABLE 3

Details of the Harmonic analysis of data in solar time from September 1961 upto March 1962 for showers with no absorber over the trays

Sl No.	Period of observation	Ist Hormonic in %	Time of Maxima	IInd Harmonic in %	Time of Maxima
1	Sept 1961	8 79	0 03 hours	5 35	7.82 19 82 hrs
2	Oct 1961	8 09	1 94 hours	5 76	3 85 15 85 hrs
3	Nov 1961	5 09	3 81 hours	4 81	5 68 17 68 hrs
4	Dec 1961	6 61	4 18 hrs	6 57	4 50 16 50 hrs
5	Jan 1962	1 58	21 74 hrs.	3 27	3 32 15 32 hrs
6	Feb 1962	1 40	5 55 hrs	1 55	5 97 17 97 hrs
7	March 1962	5 72	1 11 hrs	5 75	8 58 20 58 hrs
8.	Sept 1961 to March 1962	5 39 ± 1 2%	0 87 hrs	3 84 ± 1 2%	8 18 20 18 hrs.

than a mixed group of showers in which low energy electrons as well as μ-mesons are included in selection. In this group the significance level

Fig.(3)

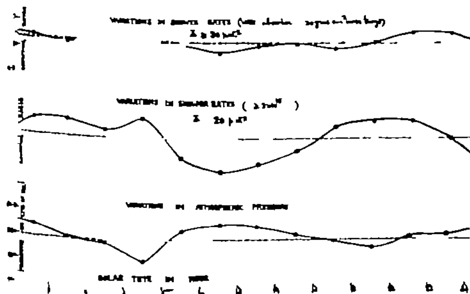


TABLE 4

Details of Harmonic analysis of the data in solar time from July 1960 upto August 1961 showers with an absorber (≥ 30 gm cm⁻²)

Sl. No.	Period of observation	Ist Harmonic Amplitude %	Time of maxima	IInd Harmonic Amplitude %	Time of maxima
1	July 1960	5.83	15.90 hrs.	1.56	0.56 12.56 hrs.
	Aug 1960	11.88	19.73 hrs.	1.5	1.13 13.13 hrs.
3	Sept 1960	3.42	21.49 hrs.	2.31	11.43 23.43 hrs.
4	Oct 1960	7.49	18.61 hrs.	5.06	10.03 22.03 hrs.
5	No 1960	2.79	14.85 hrs.	2.10	11.05 23.05 hrs.
6	Dec 1960	9.59	2.67 hrs.	3.41	11.15 23.15 hrs.
7	Jan 1961	2.07	4.78 hrs.	0.64	10.43 22.43 hrs.
8	Feb 1961	4.90	5.90 hrs.	2.04	9.52 21.52 hrs.
9	March 1961	4.80	7.45 hrs.	11.00	0.19 12.19 hrs.
10	April 1901	2.63	11.51 hrs.	5.58	4.63 16.63 hrs.
11	May 1961	6.47	2.75 hrs.	2.52	10.78 22.78 hrs.
12	June 1961	3.40	0.56 hrs.	3.78	10.34 22.34 hrs.
13	July 1961	1.77	1.53 hrs.	0.83	0.19 11.19 hrs.
14	Aug 1961	7.82	0.63 hrs.	3.96	4.96 16.96 hrs.
15	July 1960 to Aug 1961	0.61 ± 1.4%	23.60 hrs.	.51 ± 1.4%	9.87 21.87 hrs.

of the 1st Harmonic amplitude is four per cent and 2nd Harmonic amplitude is two and a half per cent. In order to give relative picture of these two groups of showers in relation to the atmospheric pressure variations the shower rates have been plotted along with a 24 hour pressure data averaged over a period of thirteen months—in Fig 5 It is evident from the nature of variations that when the pressure decreases the shower rates record an increase and when the pressure increases the shower rates record a decrease This behaviour is common in both the groups but the relative fluctuations are very much marked in the group of showers which respond to all the charged particles (i e. no absorber covers the trays)

Pressure Coefficients—Since the two groups of showers show a relatively different amplitude of fluctuation with barometric pressure, they might give different values of barometric coefficient. The data in the two groups has therefore been analysed for barometric coefficient Barometric coefficient is given by the relation $B = -\frac{d \log R}{dp}$

The logarithm of shower rates is plotted against the pressure of the atmosphere for the two groups of showers A mean straight line is drawn from the points, the gradient of this curve gives the barometric coefficient. It has been found that the pressure coefficient of the showers recorded with no absorber is $-14{\cdot}8\%$ per cm. of Hg and those recorded with an absorber over trays (>50 gm.cm^{-2}) is $-6{\cdot}7\%$ per cm. of Hg at Naini Tal

Discussion

The integral frequency energy spectrum of showers has been measured for four energy groups in the $5\times10^{14}-10^{16}$ e volt region. From the astrophysical point of view there is a great interest in this region as it provides a test for various theories of acceleration and trapping of Cosmic Ray particles in a particular region of the space. The energy of a particle spiralling in a magnetic field is given by $E=300$ ZHR e. volts, where Z is the charge of particle, R its radius of curvature and H is the magnetic field. A proton of 10^{14} e. volts energy will hardly be confined in the solar system. The energy spectrum also does not show any discontinuity at 10^{14} e. v and one has naturally to think of galactic origin of the majority of Cosmic Ray particles Inter-galactic magnetic fields are $\approx 5\times10^{-6}$ Gauss and the dimension of scattering regions $\approx 10^{19}$ cms. The primary protons of energy $>10^{16}$ e. volts will not be traped in such fields and if the sources are galactic in nature then there should be some anisotropy and a possible discontinuity in the energy spectrum at this energy Even if it is assumed that the particles are accelerated in the galactic link as well a discontinuity should be observed at 10 e volts.

From our observations of the energy spectrum at four points in this region we find that the exponent of the energy spectrum shows an increase

between 5×10^{14} and 0.89×10^{14} e volts. Its value changes from 1.71 to 2.1. This increase may be due to many reasons. The solid angle factor in the determination of flux is related with the pressure coefficient. If the pressure coefficient shows an increase, the energy exponent will also increase in this region. There is some indication that pressure coefficient gradually increases at 10^{15} e. volts. This might throw some light on the nature of interaction at ultra-high energies.

The two groups of showers that we have included in this analysis for pressure correlations show a distinctly different behaviour. The fluctuations in the shower rates above 10^{14} e volts primary energy in Solar time are of local character and have their origin in the density fluctuations of the atmospheric gases either due to shifts in various isobaric layers or due to variations of temperatures of the layers close to earth. The temperature fluctuations have relatively small effect on shower rates.

In our first group of showers where all the secondaries are allowed to trigger the G. M. counter trays, the correlation of pressure fluctuations is quite significant. The 1st and 2nd harmonic amplitudes are found to be in antiphase with the corresponding pressure harmonics and have values equal to $(5.39 \pm 1.2)\%$ and $(3.84 \pm 1.2)\%$ respectively as expected. The errors indicated here are equal to 2σ errors (i. e. $\approx 2\sqrt{\frac{2}{N}}$) where N is the total number of showers recorded. The pressure coefficient is $\approx 14.8\%$ per cm. of Hg. In this case. This value is higher than those observed by others for the same energy (i. e. $\approx 10\%$ per cm. of Hg). A large pressure coefficient has been recorded in our case due to the fact that highly inclined showers (with $\theta >$ 45°) approaching the apparatus are eliminated. The showers with large inclination to the zenith of the place of observation have to travel longer thickness in the atmosphere and mostly consist of μ-mesons which are not much influenced by atmospheric effects. Due to this the value of absorption length measured is smaller in our case i. e. ≈ 92.5 gm. cm^{-2}.

The absorption mean free path is written as $\frac{1}{\Lambda} = B = \frac{\gamma}{\lambda}$ where γ is the exponent in the integral frequency number spectrum and is ≈ 1.5 for this energy. λ is the absorption mean free path of secondary collisions of shower particles and can be determined in this case.

$$\lambda = 1.5 \times 92.5 \qquad = 138.75 \text{ gm cm}^{-2}$$

This comes out to be nearly equal to twice the geometrical collision mean free path for protons in air. This shows that the high energy collisions which generate shower particles are partly elastic and partly inelastic. The inelasticity parameter $(1-f)$ is of the order of .53. The energy retained by the nucleons in each collision as calculated from Cranshaw's (1960)[14] relation is $\approx 47\%$. The value of λ in it used is 70 gms cm^{-2}. Showers of the second group which have been selected through penetrating component give to

significant solar variations and the barometric coefficient is also very small. The pressure coefficient of this group will not represent the correct behaviour of the absorption of the nuclear active cascades. These showers however will be much helpful in finding the correct sidereal anisotropy of the primaries as these are free from solar fluctuations.

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FURTHER STUDIES ON THE KEEPING QUALITY OF CREAMERY BUTTER DURING STORAGE

MANOHAR SHYAMJI N N PANDIT AND S N SINGH

Department f Animal Husbandry & Dairying B R College Agra

In a previous paper from these laboratories (Pandit *et al* 1960) dealing with an investigation carried out to study the effect of certain factors on the keeping quality of creamery butter during storage it was reported that butter made from unripened cream but given identical treatments in respect of washing and salting kept longer at 40°F than the one from cream ripened to 0·45% serum acidity The present study is the continuat on of th same programme and is designed to study the effect of neutral sation of sour cream churning acid ty of cream and salt ng of butter on the marketab e and keeping quality of butter during storage at 40°F

METHODS AND MATERIAL

The experiment was designed to study the effect of —

(A) Varying levels of churning acidities of cream

  (i) 0·23 per cent serum acidity (fresh cream)

  (ii) 0 34 per cent serum acidity (ripened cream)

  (iii) 0·70 per cent serum a idity (ripened cream)

(B) Neutralisation of sour cream Cream testing 0 70 per cent serum acidity neutralised to 0·34 per cent

(C) Salting

  (i) No salt.

  (ii) Sa ting at 2 per cent level

E ght butter samples, seven from pasteurised ripened cream and one from pasteurised sweet cream were stored in the ref igerator at 40°F in th form of one ounce packets and were exam ned fort ightly upto a period of 98 days for the follow ng tests —

1 Organoleptic tests

  (a) Flavour

  (b) General appearanc

2. Chemical constants

   (a) Acid value

   (b) Peroxide value and

   (d) Iodine value.

3 Physical constants

   (a) Butyro-refractrometer reading

The method for the determination of different organoleptic tests were those adopted by Hunziker (1940) while for other physical and chemical constants methods recommended by A O A. C (1950) were employed.

Results and Discussion

The present investigation was planned to study a few factors like churning acidity of cream, neutralisation of sour cream and the effect of salt on the quality of butter and the various changes it undergoes during storage. The results of the investigation follow

Effect of serum acidity of cream, neutralisation of sour cream and salt content on the flavour and general appearance of butter

A perusal of the observations in table 1 will indicate that the flavour of fresh butter obtained from unripened cream was flat, whereas ripened cream butters gave a pleasant flavour That flavour development depends upon the chemical compounds formed by heterofermentative group of organisms like *L. citrovorum* and *L. dextranicum* has been shown by Bergy (1918), Badicoat (1935) and Hammer (1948) During the first fortnight sweet-cream butter continued to give a flat flavour whereas ripened cream butters developed storage flavour in second fortnight, which changed to fishy and tallowy leading finally to foul smell and rancidity after 40 days. The earlier initiation of spoilage in sweet-cream butter than in sour-cream one depicts a preservative effect of acidity on the growth of fermentation organisms. The general appearance of butter from all the lots of cream showed normal appearance in colour texture and body upto two weeks. On the 28th day examination unripened cream butter showed distinct discoloration, whereas the ripened ones showed normal colour There was a profuse mold mycelia infestation in the former case with the advance in storage time, while the latter exhibited yellow patches after 56 days and mold infestation in the final stages. The appearance of yellow spots is associated with the bleaching of fat as a result of auto-oxidation

TABLE 1

Showing the effect of churning acidity of cream on the flavour and general appearance of butter stored at 40°F

Churning Acidity of Cream	Days in Storage							
	Fresh	14	28	42	56	70	84	98
I *0.25% lactic acid*								
(a) Flavour and aroma	Very mild	Very mild	Flat	Fishy	Tallowy	Rancid	Ext rancid	Rancid foul smell
(b) General appearance	Good	Good	Discolouration	Mouldy	Mouldy	Black mould	Mouldy	Mouldy
II. *0.34% lactic acid*								
(a) Flavour and aroma	Diacetyl	Diacetyl	Diacetyl	Flat	Stale	Slightly fishy	Fishy	Tallowy
(b) General appearance	Good	Good	Good	Bleached	Yellow spots	Black & Yellow spots	Black & Yellow spots	Mouldy
III *0.70% lactic acid*								
(a) Flavour and aroma	Pleasing diacetyl	Acidic	High acidic	Slightly storage & acidic	Stale	Stale	Slightly fishy	Fishy
(b) General appearance	Good	Good	Bleached	Bleached	Bleached	Bleached	Mouldy	Mouldy
IV *Neutralised cream 0.34% lactic acid*								
(a) Flavour and aroma	Trace of neutraliser	Neutraliser	Storage	Storage	Stale	Stal	Stale	Fishy
(b) General appearance	Good	Good	Good	Bleached	Shrinked	Shrinked	Shrinked	Shrinked

TABLE 2

Showing the effect of salt on the flavour and general appearance of butter stored at 40° F

Churning Acidity of Cream	Days in Storage							
	Fresh	14	28	42	56	70	84	98
Sweet Cream 0.3% lactic acid								
(a) *Salted*								
(i) Flavour and aroma	Mild	Mild	Slight flat	Fishy	Fishy	Tallowy	Rancid	Rancid
(ii) General ppearance	Good	Y llow colou	Discolouration	Mould Specks	Mould Specks	Mouldy	Mouldy	Mouldy
(b) *Unsalted*								
(i) Flavour and aroma	Very mild	Very mild	Flat	Fishy inedible	Tallowy	Rancid	Extremely rancid	Rancid & Foul
(ii) General appearance	Good	Yellow colour	Discolouration	Mould spots	Mouldy	Black & brown moulds	Mouldy	Mouldy
Mild acid cream 0.34% lactic acid								
(a) *Salted*								
(i) Flavour and aroma	Excellent & pleasing	Diacetyl	Diacetyl	Slight flat	Slight tale	Stale	Fishy	Fishy
(ii) General appearance	Good	Y llow colour	Yellow colou	Discolouration	Y llow spots	Blackmould buttons	Surface mould	Surface mould
(b) *Unsalted*								
(i) Flavour and aroma	Desirable	Desirable	Desirable	Flat	Stale	Slightly fishy	Fishy	Tallowy
(ii) General ppearance	Good	Light yellow colour	Ligh yellow colour	Bleaching	Yellow spots	Black spot and yellowish	Surface mould	Surface mould

(Continued on page [illegible])

TABLE 2 (Contd.)

Showing the effect of salt on the flavour and general appearance of butter stored at 40° F

Churning Acidity of Cream	Days in Storage							
	Fresh	14	28	42	56	70	84	98
Sour cream 0·70% lactic acid								
(a) *Salted*								
(i) Flavou and aroma	Pleasing diacetyl	Pleasing diacetyl	Pleasing acidic	Storage	Storage	Stale	U pleasant	Fishy
(ii) General appearance	Good	Deep yellow colour	Deep yellow colour	Bleaching	Stal	Stale	Stale	Slight mouldy
(b) *Unsalted*								
(i) Flavour and aroma	Pleasing diacetyl	Acidic	High acidic	Storage	Stale	Stale	Slightly fishy	Fishy
(ii) General appearance	Good	Good	Fading colour	Bleaching	Bleaching	Bleaching	Bleaching	Mouldy
Neutralised cream 0·31% lactic acid (Neutralised from 0·70 to 0·31% lactic acid)								
(a) *Salted*								
(i) Flavour and aroma	Trace of neutraliser	Good	Storage	Storage	Flat	Stale	Stal	Fishy
(ii) General appearance	Good	Deep yellow collou	Deep yellow colour	Bleaching	Bleached	Bleached	Bleached	Bleached
(b) *Unsalted*								
(i) Flavour and aroma	Trace of neutraliser	Trace of neutraliser	Storage	Storage	Flat	Stale	Stale	Fishy
(ii) General appearance	Good	Light yellow colour	Light yellow colour	Bleaching	Discoloured	Discoloured	Discoloured	Discoloured

According to Hammer (1948) mold being aerobic causes colour defects on the surface and eventually produces discoloration around the butter. Neutraliser imparted its own flavour to butter samples which lasted even during the storage although it inhibited different types of chemical deterioration. Distinct variations in flavour were recorded between salted and unsalted samples obtained from differently treated creams (Table 2). Salted samples were superior to unsalted ones while fresh and even during storage upto a fortnight probably because of the inhibiting effect of high osmotic concentration of salt on organic life. Addition of salt in high acid-cream butter did not lead to any advantage. However salt and neutraliser both showed a cumulative effect to enhance the keeping quality. During later periods of storage salt enhanced the chemical reactions decomposing lecithin particularly in high-acid-cream butters. While unsalted samples maintained a normal colour the salted ones manifested a deeper colour due to salting-out action on the curd particles (Kothawala 1937).

Effect of acidity of cream, neutralization of high-acid-cream and salt per cent on the acid value of butter

A positive correlation between the acid value of butter and the serum acidity of cream is shown in table 3 and fig 1. The acid values observed for 0.23, 0.34 and 0.70 per cent serum acidity were 0.27, 0.36 and 0.45 respectively. A uniform increasing trend in the acid value of butter was noted during storage, which agrees with the findings of Larsen et al. (1909).

TABLE 3

Showing the effect of churning acidity of cream on the acid value of butter stored at 40°F

Churning acidity of cream	Days in Storage							
	Fresh	14	28	42	56	70	84	[illegible]
0.23 lactic acid								
(a) Acid value as oleic	0.27	0.42	0.60	0.76	0.83	0.93	1.01	1.05
(b) Per cent rate of increase	–	55.5	42.8	26.6	13.1	10.4	6.3	3.3
0.34 lactic acid								
(a) Acid value as oleic	0.36	0.60	0.84	1.05	1.14	1.22	1.30	1.35
(b) Per cent rate of increase		66.6	40.0	25.0	8.5	7.0	6.5	1.5
0.70 lactic acid								
(a) Acid value as oleic	0.45	0.79	1.02	1.13	1.18	1.20	1.22	1.25
(b) Per cent rate of increase		75.5	29.1	10.7	4.4	1.6	1.6	[illegible]
0.34 lactic acid neutralised cream								
(a) Acid value as oleic	0.33	0.54	0.69	0.78	0.81	0.84	0.87	0.8[illegible]
(b) Per cent rate of increase		63.6	27.7	13.0	3.8	3.7	3.5	2.5

TABLE 4

Showing the effect of churning acidity of cream and salt % on the acid value of butter stored at 40° F

Churning acidity of cream	Days in Storage							
	Fresh	14	28	42	56	70	84	98
Sweet Cream 0.25% lactic acid								
(a) *Salted*								
(i) Acid value % oleic	0.24	0.36	0.51	0.65	0.74	0.82	0.87	0.90
(ii) % rate of increase	...	50.0	41.6	27.4	13.8	10.8	6.0	3.4
(b) *Unsalted*								
(i) Acid value % oleic	0.27	0.42	0.60	0.76	0.85	0.95	1.01	1.05
(ii) % rate of increase	...	55.5	42.8	26.6	13.1	10.4	6.3	3.9
Variation *	0.03	0.06	0.09	0.11	0.11	0.14	0.14	0.15
Mild Acid Cream 0.34% Lactic Acid								
(a) *Salted*								
(i) Acid value % oleic	0.34	0.56	0.78	0.90	1.02	1.14	1.26	1.26
(ii) % rate of increase	...	64.7	39.2	15.3	13.3	11.7	10.5	0.0
(b) *Unsalted*								
(i) Acid value % oleic	0.36	0.60	0.84	1.05	1.14	1.22	1.30	1.32
(ii) % rate of increase	...	66.6	40.0	25.0	8.5	7.0	6.5	1.5
Variation *	0.02	0.04	0.06	0.15	0.12	0.08	0.06	0.06
Sour Cream 0.70% lactic Acid								
(a) *Salted*								
(i) Acid value % oleic	0.42	0.72	0.82	1.02	1.06	1.08	1.09	1.10
(ii) % rate of increase		71.4	27.7	10.8	3.9	1.8	0.9	0.9
(b) *Unsalted*								
(i) Acid value % oleic	0.45	0.79	1.02	1.13	1.18	1.20	1.22	1.22
(ii) % rate of increase	...	75.5	29.1	10.7	4.4	1.6	1.6	0.0
Variation *	0.03	0.07	0.10	0.11	0.12	0.12	0.13	0.12
Neutralized cream 0.34% Lactic acid								
(a) *Salted*								
(i) Acid value % oleic	0.30	0.48	0.60	0.72	0.75	0.78	0.81	0.84
(ii) % rate of increase		60.0	25.0	20.0	4.1	4.0	3.8	3.7
(b) *Unsalted*								
(i) Acid value % oleic	0.33	0.54	0.69	0.78	0.81	0.84	0.87	0.89
(ii) % rate of increase	...	62.6	27.7	13.0	3.8	3.7	3.5	2.2
Variation	0.03	0.06	0.09	0.06	0.06	0.06	0.05	0.03

The percentage increase in acid value went on declining during storage, possibly because some of the free fatty acids must have been consumed by yeasts and moulds Similar results were observed by Rangappa and Achaya (1948) for butter and ghee. Neutralised acid-cream butter exhibited lower acid values. The values went down by 26·66 per cent from the base value. The addition of salt (Table 4) was found to have a marked effect in the elimination of butter-milk from butter thus lowering down its acid value. During the storage period of 98 days the acid values of butter obtained from sweet cream reached upto 1·05 and 0·90 for unsalted and salted samples respectively Thus clearly denotes an antiseptic property of salt against spoilage organisms.

Effect of acidity of cream neutralisation of sour cream and salting on the peroxide value of butter during storage

The peroxide values of butter prepared from different acid creams are shown in table 5 and fig 2 The values observed from 0·23 0·34 and 0·70 per cent acid-cream butters were 0·22 0·42 and 0·63 respectively. These values increased with the increasing storage periods. The rate of increase in peroxide values was higher in sweet-cream than in sour cream butters The lower peroxide values in mild-acid-cream butters indicate a slower rate of oxidation in them than the high-acid-cream butters and thus shows an inverse relationship between the peroxide value and the quality of butter These results support the findings of Sommer (1937) The rapid rise in the peroxide value during storage is an evidence of the oxidising conditions as suggested by Davies (1939) Neutralised cream butter showed about 12·0 per cent lower values than mild-acid-cream butter and this trend remained the same even during the storage period Salted butters exhibited higher peroxide values than unsalted samples (Table 6) It seems that salt accelerates the oxidation of butter fat. It dissolves phospholipid layer and exposes the fat for ready oxidation

Effect of acidity of cream neutralisation of sour Cream and salting of butter on the physical score of the product

The scores of different classes of butter given in table 7 reveal that mild acid-cream butter (0.34 per cent lactic acid) scores the highest—95 followed by high-acid-cream. The sweet-cream butter score the least—90 when fresh, followed by the neutralised cream butter—88. During storage, sweet-cream butter deteriorated earlier than other types. The better initial higher score in case of acid cream is obviously due to the flavour and aroma produced during ripening by the break down of citric acid and lactose High-acid-cream butter (0 70 per cent) showed a more rapid decline in score than mild and neutralised samples in the earlier stages, but ran parallel to them later on. Salting as seen from table 7 adds no advantage in the score of fresh butter During storage however salting is able to maintain a uniformly higher score.

Effect of acidity of cream, neutralisation of sour cream and salting of butter on B R. and Iodine values of butter

Examination of data in table 8 depicts a slow and continuous but irregular rise in B. R. reading of butter fat. During storage there was a fall in values irre pective of the treatment involved. The rise in B R. value is due to the replacement of part of the acid radical of glyceride molecule with the hydroxyl group known to possess greater refractive indices. These observa tions quite agree with those of Richmond (1942) and Godbole and Sadgopal (1939)

Table 9 gives an almost constant iodine value—33 for all the samples upto 28 days of storage with a subsequent fall with the passage of time. The decrease in iodine value by 13·02 per cent during the storage period in all the samples can be attributed to the oxidative changes taking place during storage. It seems likely that oleic acid of which iodine value is a measure, is oxidised giving rise to hydroxy-acids resulting in poorer iodine absorbing capacity Achaya and Banerjee (1946) and Richmond (1942) reported a similar tendency which conflicts with the view held by Das Gupta (1949) who reported an increase in iodine value during storage

TABLE 5

Showing the effect of churning acidity of cream on the peroxide value of butter stored at 40°F

Churning acidity f cream	Days in Storage							
	Fresh	14	28	42	56	70	84	98
Sweet Cream 0.25% lactic acid								
( ) Peroxide value	0 22	1 8	2 4	3 2	4 0	4 8	5 6	5 8
(b) % rate of increase		718 1	33 3	33 3	25 0	20 0	16 6	3 4
Mild acid cream 0.34% lactic acid								
( ) Peroxide value	0 42	2 2	3 2	3 7	3 9	4 0	4 1	4 2
(b) % rate of increase		423 8	45 0	15 6	5 4	2 5	2 5	2 4
Sour cream 0.70% lactic acid								
(a) Peroxide value	0 63	3 2	4 5	5 4	5 5	5 6	5 7	5 8
(b) % rate of increase		407 8	40 6	20 0	1 8	1 8	1 7	1 7
Neutralised cream 0.34% lactic acid								
( ) Peroxide value	0 37	2 3	3 3	3 8	4 0	4 1	4 2	4 3
(b) % rate of increase		521 6	43 4	15 1	5 2	2 5	2 4	2 3

TABLE 6

Showing the effect of salt on the peroxide value of butters stored at 40° F

Churning acidity of cream	Days in Storage							
	Fresh	14	28	42	56	70	84	98
Sweet Cream 0.23% lactic acid								
(a) *Salted*								
(i) Peroxide value	0 28	2 2	2 8	3 4	4 1	4 9	5.7	[illegible]
(ii) % rate of increase	...	683 7	27 2	21 4	20 5	19.5	16 3	5.3
(b) *Unsalted*								
(i) Peroxide value	0 22	1 8	2 4	3 2	4 0	4.8	5.6	[illegible]
(ii) % rate of increase	...	718 1	33 3	33 3	25 0	20 0	16 6	3.4
Variation*	0 06	0 40	4 0	0.2	0 1	0 1	0 1	[illegible]
Mild acid cream 0 34% lactic acid								
(a) *Salted*								
(i) Peroxide value	0 44	2 7	3 8	4 0	4 2	4 4	4 5	4 6
(ii) % rate of increase	..	513 6	40 7	5.2	5 0	4 7	2.2	[illegible]
(b) *Unsalted*								
(i) Peroxide value	0 42	2 2	3 2	3 7	3.9	4 0	4 1	4
(ii) % rate of increase	..	423 8	45 0	15 6	5 4	2 5	2.5	[illegible]
Variation*	0 02	0 5	0 6	0.3	0 5	0 4	0 4	[illegible]
Sour Cream 0 70% lactic acid								
( ) *Salted*								
(i) Peroxid value	0 68	3 6	5 0	5 6	5.8	6 0	6.2	6 3
(ii) % rate of increase	...	429 4	38 8	1.20	3 5	3 4	3 3	1 6
(b) *Unsalted*								
(i) Peroxide value	0 63	3 2	4 5	5 4	5 5	5 6	5 7	[illegible]
(ii) % rate of increase	...	407 8	40 6	20 0	1 8	1.8	1 7	1 7
Variation*	0 05	0 4	0 5	0 2	0.3	0 4	0 5	[illegible]
Neutralised cream 0.34% lactic acid								
( ) *Salted*								
(i) Peroxide value	0 40	2 8	3 8	4 1	4 4	4 6	4 8	[illegible]
(ii) % rate of increase		600 0	35 7	7.8	7 3	4.5	4 3	[illegible]
(b) *Unsalted*								
(i) Peroxide value	0 37	2 3	3 3	3 8	4.0	4 1	4 2	4 3
(ii) % rate of increase	...	521 6	43 4	15 1	5.2	2 5	2 4	2 3
Variation*	0 03	0 5	0 5	0.3	0 4	0.5	0.6	[illegible]

TABLE 7

Showing the effect of Churning acidity of cream and salt per cent on the Score of butter stored at 40° F

Churning acidity of cream	Days in Storage							
	Fresh	14	28	42	56	70	84	98
Sweet cream 0.23% lactic acid								
(a) *Salted*								
(i) Score %	92	88	82	70	60	45	40	35
(ii) Rate of decrease %		4 5	7 3	17 1	16 1	53 3	12 5	14 2
(b) *Unsalted*								
(i) Score %	90	85	78	62	55	40	36	30
(ii) Rate of decrease %	...	5 8	8 9	9 6	14 0	57 5	11 1	20 1
Mild acid cream 0.51% lactic acid:								
( ) *Salted*								
(i) Score %	96	94	92	85	80	70	60	50
(ii) Rate of decrease %	..	2 1	2 1	8 2	6 2	14 2	16 6	20 0
(b) *Unsalted*								
(i) Score %	95	92	88	81	75	65	55	44
(ii) Rate of decrease %	..	3 2	4 5	8 6	8 0	15 3	18 1	25 0
Sour Cream 0.70% lactic acid:								
a) *Salted*								
(i) Score %	99	92	84	78	72	60	55	43
(ii) Rate of decrease %		7 6	8 5	7 6	8 3	20 0	9 0	22 2
(b) *Unsalted*								
(i) Score %	92	90	85	82	72	62	58	46
(ii) Rate of decrease %		2 2	5 8	3 6	13 8	16 1	6 8	26 0
Neutralised cream 0.35% lacti acid								
(a) *Salted*								
(i) Score %	91	90	86	82	80	72	60	52
(ii) Rate of decrease %		1 1	4 6	4 8	2 5	11 1	16 1	19 2
(b) *Unsalted*								
(i) Score %	88	86	84	80	76	66	60	50
(ii) Rate of decrease %	...	2 3	2 3	5 0	5 2	15 1	10 6	20 0

TABLE 8

Showing the effect of Churning acidity of cream, neutralisation of sour cream and salting on the B. R. readings of butter stored at 40° F

Churning acidity of cream	Days in Storage: Fresh	14	28	42	56	70	84	98
Sweet Cream 0.23% lactic acid								
(a) Salted	42.50	42.55	42.60	42.65	42.75	42.50	42.50	[illegible]
(b) Unsalted	42.30	42.60	42.60	42.70	42.80	42.84	42.85	[illegible]
Mild acid cream 0.34% lactic acid:								
(a) Salted	42.50	42.50	42.55	42.60	42.75	4[illegible].80	42.85	[illegible]
(b) Unsalted	42.50	42.50	42.60	42.65	42.85	42.88	42.90	[illegible]
Sour cream 0.70% lactic Acid:								
(a) Salted	42.50	42.55	42.65	42.85	42.90	42.90	42.95	[illegible]
(b) Unsalted	42.50	42.60	42.70	[illegible].85	42.90	42.93	42.95	[illegible]
Neutralised cream 0.34% lactic acid:								
(a) Salted	42.50	42.50	42.55	42.60	42.80	42.85	42.85	[illegible]
(b) Unsalted	42.50	42.30	42.55	42.65	[illegible].80	42.85	42.90	[illegible]

TABLE 9

Showing the effect of Churning acidity of cream, neutralisation of sour cream and salting on the iodine value of butter stored at 40°F

Churning acidity of cream	Days in Storage: Fresh	14	28	42	56	70	84	98
Sweet cream 0.23% lactic acid								
( ) Salted	33.02	33.02	33.02	32.50	30.50	30.60	29.20	[illegible]
(b) Unsalted	33.02	33.02	33.02	32.50	30.50	[illegible]	29.40	[illegible]
Mild acid cream 0.34% Lactic acid								
( ) Salted	33.02	33.02	33.02	32.50	30.50	[illegible].55	[illegible]	[illegible]
(b) Unsalted	33.02	33.02	31.02	32.50	30.70	29.45	[illegible]	[illegible]
Sour cream 0.70% lactic acid								
( ) Salted	33.02	33.02	33.02	32.50	30.50	[illegible].70	[illegible]	[illegible]
(b) Unsalted	33.02	33.02	33.02	32.50	30.70	[illegible].45	[illegible]	[illegible]
Neutralised cream 0.34% Lactic acid								
( ) Salted	33.02	33.02	33.02	32.50	30.70	[illegible]	[illegible]	[illegible]
(b) Unsalted	33.02	33.02	33.02	32.50	30.[illegible]	29.70		

Summary

The present study was planned to study the quality of butter with special reference to

(i) Churning acidity of cream,
(ii) Neutralisation of sour cream and
(iii) Salting of butter

In all there were eight samples with different treatments which were examined at fortnightly intervals for different organoleptic, physical and chemical constants The results indicate that acid-cream butters give more desirable flavour and aroma than sweet cream butter Neutralisation of high-acid cream also produced butter with lower but constant score than sweet cream. Acid values in the initial stages were proportional to the churning acidities and remained so throughout the storage period. During later stages, however there was noted a progressive decline which was more rapid in sour and neutralised cream butters than in sweet-cream one. Peroxide values also were proportional to churning acidities but exhibited a fairly low per cent rate of increase during storage

No significant effect of neutralisation could be marked on the acid value or peroxide value of butter during storage.

Salted butters produced fine flavour and remained in good condition for a longer period than the unsalted ones The effect of salt was specially marked in case of mild-acid-cream butter in enhancing its keeping quality

It can be inferred, therefore that butter manufactured from ripened cream—0·34 per cent and salted at 2 0 per cent kept better for a longer period (56 days) with better score. Neutralised high-acid-cream butter with 2·0 per cent salt kept better upto 42 days and was found to be advantageous for short storage periods. Salt has an additive effect in preserving the product for longer duration.

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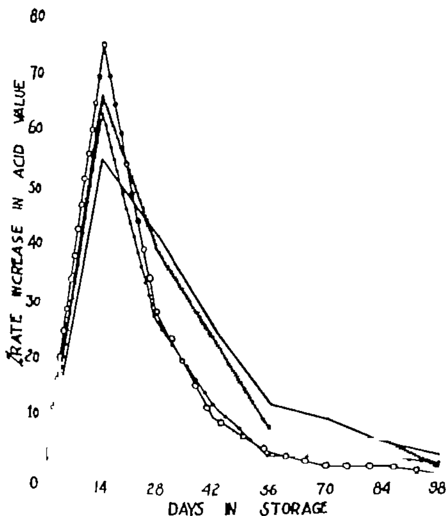


Fig. 1 Showing the effect of acidity of cream on the acid value of butter stored at 40° F

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ECOLOGICAL STUDIES ON *CAMPONOTUS COMPRESSUS* (FABR.) (FORMICIDAE HYMENOPTERA) DURING SPRING IN HARDWAR*

G S GUPTA

Head of the Zoology Department College of Science Gurukul Kangri Hardwar
Honorary Research Scholar School of Entomology St. John's College Agra

INTRODUCTION

Camponotus compressus (Fabr) is the common black ant of India. Rothney (1889) Wroughton (1892) and Hingston (1923) have given interesting but scanty accounts of the habits of this ant. Its great economic value in connection with the lac insect has been emphasised by Negi, Misra and Gupta (1930) Ayyar has regarded it as definitely harmful because of its symbiotic relationships with a number of harmful insects Ayyar (1935 and 1937) further investigated its biology nuptial flight and colony formation under climatic conditions of Coimbatore To Bingham (1903) we are indebted for bringing together works of various taxonomists on this very interesting group of insects in the form of a fauna volume. Ecology of this ant in the climatic conditions of Northern India, however has not yet attracted the attention of Indian entomologists This paper contains briefly the results of ecological investigations carried out in the campus of Gurukul Kangri Hardwar in Uttar Pradesh

GEOGRAPHICAL SITUATION AND CLIMATIC CONDITIONS OF HARDWAR

Hardwar is situated within the subtropical region Lat 29 58 N Long 78 13 E at an altitude of 965 feet above mean sea level and stands on the right bank of the river Ganga at the foot of the Siwalik or the foot hill of Himalaya. Winters are rather extreme but summers relatively moderate Actual weather data recorded at Roorkee Lat. 29.51 N Long 77 59 E at an altitude of 857 feet above mean sea level about 16 miles southwest of Hardwar would roughly represent the conditions at this place. The data collected during 1961 is as follows —

Average maximum temperature	43 7°C
Average minimum temperature	4·0°C
Average rainfall	49 19 inches

DESCRIPTION OF THE LOCALITY AND STATION

The observations were recorded from March 1962 onwards till about the third week of May 1962 at an ant nest situated at the base of an Arjuna

* Contribution No. 89 from the School of Entomology S John's College Agra.

tree *Terminalia arjuna* in the Ayurvedic College garden of Gurukul Kangri about two miles to the southwest of Hardwar railway station. The nest was situated at an exposed and windy eastern face of the tree trunk with the following measurements taken on 22 3 62 —

1	Nest hole dimensions	1 ×1
2	Depth of the nest hole as felt by introducing a soft grass stem into it, beyond which it sloped steeply into the galleries	3·0″
3	Height of the debris dug out by the ants	6·0″ on the west gradually sloping to the level ground towards north-east around the tree trunk to a distance of two feet

Diel Activity

Figure 1 and table 1 record the average activity observed on alternate days at different hours of the day and night during the months under observation Activity unit has been taken up as the number of ants counted near the nest hole or on the tree trunk upto a height of about 10 feet from the ground within a period of two minutes. As would be clear from the table and the graph, minimum activity is found at midday and peak is attained at night between 8 p m (20 hours) and 4 a.m (4 hours) Wroughton (1892) believed the ant to be crepuscular Hingston (1923) diurnal and Ayyar (1935) regards it as active throughout the day

Table I

Statement showing the average activity of Camponotus compressus at different hours during March April and May 1962 and the average for the whole season.

Months	Hours					
	12	18	20	22	4	6
March ..	2	6	23	25	21	11
April	2	5	8	4	5	7
May	3	12	16	29	21	15
Total of 3 months ..	7	23	47	49	51	33
Average of 3 months	2	8	16	16	17	11

It is significant to note that during April a marked fall in the activity is found This is perhaps due to the fact that the tree loses much of its

foliage and the ants are not able to find their food and honey-dew from their "cows the Membracids With the arrival of fresh foliage and inflorescence during May a sharp rise in activity is observed Almost during every hour of the day stray ants have been observed wandering about far from their nest These journeys are probably undertaken in search of food. They exhibit a well pronounced negative reaction to dazzling day light and artificial torch light. This behaviour seems to be at the back of their peak activity during dark hours.

Temperature and Activity

Figure 2 and table 2 show that conditions of temperature inside the nest through the season under observation remain almost uniform and exhibit very insignificant fluctuations. The nest therefore offers a constantly favourable situation where the ants retire whenever the atmospheric temperature proves intolerable. The data collected points in no uncertain terms a definite bearing of the effect of temperature on the ants activity

Table 2

Statement showing the average temperatures in °C in the air and inside the nest of C compressus during the months of March April and May 1962 and the average temperatures for the whole period.

Months		Hours 12	18	20	22	4	6
March	Air	24	20	19	17	10	13
	Nest	21	20	20	19	18	17
April	Air	35	29	27	25	19	22
	Nest	27	26	26	27	25	24
May	Air	38	33	29	26	22	25
	Nest	27	27	26	26	25	24
Total	Air	97	82	75	68	51	62
	Nest	75	73	72	72	68	65
Average of three months	Air	32	27	25	23	17	21
	Nest	25	24	24	24	23	22

During the month of April the temperature fluctuations do not seem to effect the activity to a greater extent, probably due to the lack of foliage.

In the months of March and May the effect of temperature changes on the activity is much pronounced. It would be clear from the above-mentioned figure and table that the activity varies inversely as the change of temperature. The maximum temperature is recorded at 12 hours when the activity is at the lowest level. The temperature declines gradually by 18 hours with a gradual rise in activity After 18 hours there is a steep decline in the temperature in consequence of which we find a sudden spurt in the activity. The minimum temperature is attained at about 4 hours around which time the peak of activity is reached. This however does not mean that the range of maximum activity is spread over almost the whole of the night from 20 hours to 4 hours when the temperatures are low The nocturnal nature of the ant therefore is perhaps partly due to the peculiar conditions of temperature prevailing at different hours of the day and night

Wind and Activity

Observations recorded on the effect of wind upon the activity of the ant show that the ants are on an average very active when the movement of *air is very slow* With increase *in wind velocity* the activity falls. The increase in wind velocity modifies the effect of temperature on the activity by stepping up the rate of evaporation which the ants seem to avoid and under the influence of which they retire to their nest. Apart from the risk of evaporation the danger of being blown away by the fast wind also to some extent restricts their movements

Rainfall and Activity

Highest points of activity have been recorded during clear nights The overcasted sky and the rains immediately drive the ants to their nests. It is generally believed that an onset of rains marks an increase in the activity This probably relates to the attainment of sexual maturity when the sexuals emerge from their nuptial flight During the spring season, however when the sexuales are immature the rains do not seem to stimulate the activity This is apparent here that it is not the rains which are at the back of the increased activity but the altered physiological condition of the individuals due to the maturity of the gonads.

Summary

1 Detailed investigations on the effect of temperature wind velocity and rain on the daily activity of the common Indian black ant *Camponotus compressus* are discussed.

2. The rise in temperature is followed by a marked fall in the rate of activity Maximum activity is observed when the temperature is lowest and particularly during the night.

3. Increase in wind velocity has an adverse effect upon the activity of the ant.

4. Rains during the spring instead of stepping up the activity as generally believed retard their movement.

Acknowledgments

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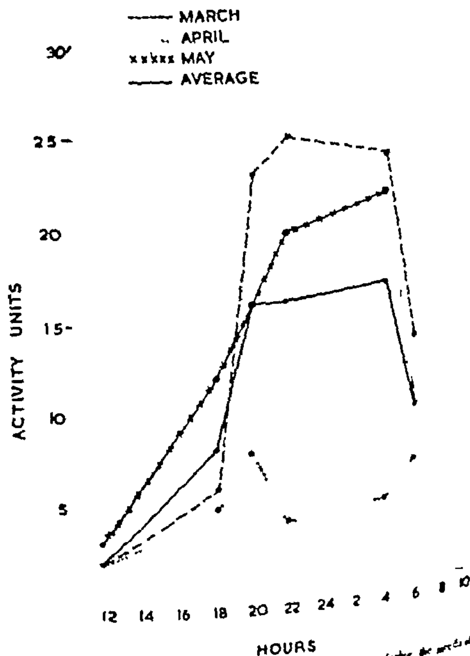


Fig. 1 Graph showing the activity of *Camponotus compressus*, during the months of March April and May 1962 at various hours of the day.

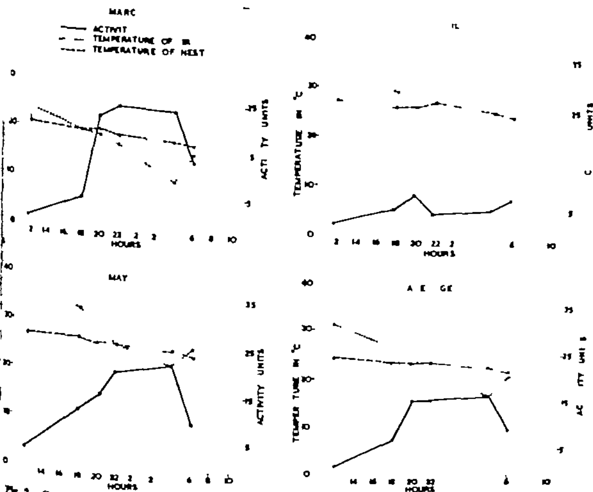


Fig. 2. Graphs showing th activity of *Camponotus compressus* t various hours of the day in relation to air and nest temperatures, during the months of March April and May 1962.

GIBBERELLIC ACID INDUCED PARTHENOCARPY IN THE PEACH

AYODHYA PRASAD

Department of Botany Govt. Agricultural College Kanpur

Recent researches revealed (1,2 8—12) the parthenocarpic development of fruits in several P unus species In the almond, apricot, Fay Elberta peach and plums parthenocarpy was induced by the application of GA (1) In previous, preliminary tests, repeated applications showed GA to be effective in promoting parthenocarpy in the self fertile varieties of peach. In fact the yields from fertile varieties are also poor when environmental conditions are conducive to inadequate pollination GA was tested in 1960 to determine if satisfactory results might be produced parthenocarpically

MATERIAL AND METHOD

Eight year old bearing Sharbati trees were selected for this study and were situated between Sharbati and Elberta varieties of peach. Aqueous solution of Potassium salt of Gibberellic Acid of 150 250 500 750 and 1000 ppm plus 0 05 per cent surfactant (Polyoxy ethylene Sorbitan monolaurate) were applied as a drench spray at full bloom stage on February 26 to emasculated blossoms. Each treatment was applied to a branch bearing from 240–435 emasculated blossom on each of 4 trees

Twenty blossoms on each branch were tagged The fruit set percentage was recorded at maturity At harvest 30 fruits of each treatment were selected at random and used for recording average cheek diameter soluble solids and titrable acids. Results are summarized in the table 1 below

TABLE 1

Effect of GA on the percentage of parthenocarpic set, cheek diameter soluble solids and titr ble acids of Sharbati peach at maturity *

Concentration of GA parts per million	Fruit set %	Cheek diameter cm.	Soluble solids %	Titrable acids ml. equivs.
150	43 6	4 55	10 5	0 395
250	50 4	4 69	10 4	0 386
500	61 5	4 84	10 1	0 365
750	52 3	4 86	10 7	0-3.2
1000	44 2	4 95	10 6	0 340
Emasculated only	11 4	3 76	7 8	3 342
Open pollinated	9 5	6 27	8 9	0 309

* Fruit set was recorded after one month of spray

It is clear from table 1 that GA induced parthenocarpic fruits, ranging from 43 6 to 61 5 per cent, which were appreciably greater than the normal fruits of open pollinated branches. All parthenocarpic fruits were elongated and looking normal in appearance however they were smaller in diameter than pollinated fruits. Cheek diameter increased progressively as GA concentration was increased.

The fruits were compared in respect of the dimension and flesh thickness of 50 fruits of about same cheek diameter from each treatment. The measurements of mature GA induced parthenocarpic fruits and immature pollinated fruits were made on June 12, and the results are recorded in table 2.

TABLE 2

Effect of GA on Fruit and flesh dimensions of GA—induced parthenocarpic Sharbati peaches.

Treatment (GA in ppm)	Fruit			Flesh thickness		
	Cheek diameter cm.	Suture Diameter cm.	Length cm.	Cheek cm.	Suture cm.	Stem or Blossom end cm.
150	4 61	4 55	6 72	2 31	1·31	1·48
250	4 63	4 62	6 78	2 43	1 33	1 58
500	4 78	4 74	6 81	2 65	1 43	1·47
750	4 90	4 85	7 30	2·92	1·65	1 59
1000	4 96	4 86	7 55	3·00	1·82	1 68
Open pollinated (Immature)	4 85	4 80	6 08	2·06	1·00	1 57
Open pollinated (mature)	5 98	5 75	6 43	3 45	3 51	1 71
Emasculated only	3 18	4 10	5 06	1 81	1 20	1 52

It is evident from Fig 1 and *table 2 that GA* increased growth along the longitudinal axis of fruits. The GA induced fruits were not only greater in length but also thicker in flesh at the stem and blossom ends. The soluble solids as well as colour and firmness indicated that the parthenocarpic fruits ripened a week earlier than pollinated fruits. *GA induced fruits of several* species of prunus have been *reported to ripe* in advance of pollinated fruits (2 6) Titrable acids in GA induced fruits were greater than the pollinated ones but a trend of decreasing acidity was found with the increasing of GA concentrations.

Discussion

Parthenocarpy has been induced in several fruits by auxin application and the research workers (4 9 11 and 12) concluded that auxin is the limiting factor in the setting and subsequent growth of fruits Phinney et al (7) found Gibberelin like substance in the young seeds of several prunus species. Gustafson (3) reported that auxin content was greater in naturally parthenocarpic ovaries than in their non parthenocarpic counterparts. Since GA induced parthenocarpy in the almond apricot and peach Crane et al (1) suggested that GA or GA-like substances also should be considered essential for fruit set and development. Small undeveloped fruites may be presumed by partial lack of such growth stimulus. In case of cherry (9) GA plus an auxin induced parthenocarpic fruits while either of them failed in this respect.

Primer and Crane (8) pointed out that the stimulus is necessary for cell division and cell enlargement in parthenocarpic apricots induced with auxin treatment. For the parthenocarpic fruits had less layers of smaller cells and consequently the average diameter of these cells was only 40% of that of fertilized fruits. Small sized peaches in view of cell number irregular shape and uneven maturity would be seen to be comparable to parthenocarpic apricots produced by treatment with the auxin 2 4,5 —Trichloro-phenoxyacetic acid (8) The stimulus may deficient in Sharbati small sized peaches which is apparently necessary for normal cell division and enlargement in the mesocarp appears to be GA or a GA-like material. This is indicated by the fact that not a single fruit of parthenocarpic type was produced on any of the branches unsprayed and pollinated.

The development of endosperm depends upon the auxin content of the ovule. Stahly and Thompson (10) are of similar opinion that the highest auxin content in Halen haven peach ovules is associated with rapid development of the endosperm and embryo and with the slowest growth of the mesocarp. Luckwill (4) points out that once initiation has occurred subsequent growth very soon comes under the control of auxin which has its origin within the developing seed.

According to Luckwill (5) at least two kinds of chemical stimuli are involved in the fruit development—primary stimulus initiates ovary growth while secondary stimulus originating in the seeds, is necessary fo the continued growth to maturity Thus it seems that the factor responsible for the development of fruit growth is inherent. Once the primary stimulus is operative through pollination or the application of auxin—GA, the growth phenomenon of the resultant fruit is similar to Cherry (9) and pear (6)

Summary

1 Aqueous solutions of GA 150 250 500 750 and 1000 ppm applied as spray to emasculated Sharbati peach blossom promoted parthenocarpic fruit set of from 43.6 to 61.5 per cent.

2 The parthenocarpic fruits were smaller in diameter than p ... ones but they were larger in flesh at the stem and bl... om ends.

3 Titrable acids decreased with the increase in GA concentrations. H... ever the titrable acids and soluble solids were increased over ... control

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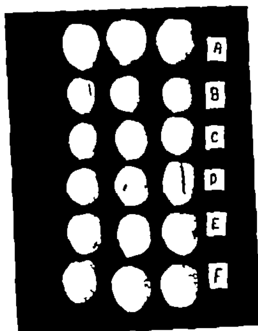


Fig 1 A—Open pollinated and B—150 C—250, D—500, E—750, F—1000 ppm. parthenocarpic fruits of Sharbati Variety of peach at maturity

2 The parthenocarpic fruits were smaller in diameter than pollinated ones but they were larger in flesh at the stem and blossom ends

3 Titrable acids decreased with the increase in GA concentrations. However the titrable acids and soluble solids were increased over the control

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STUDIES ON THE HEART OF *CLARIAS MAGUR* (CUV AND VAL.)

E. I THOMAS
Department of Zoology Christ College Irinjalakuda Kerala State

INTRODUCTION

Clarias magur is a fresh-water teleostean fish available fairly in large number all the year round in various parts of South India. It is studied as a representative type of the siluroid fish in various colleges There does not, however exist any published anatomical account of this fish. It is, therefore, very essential to have a detailed knowledge of the anatomy of this fish. The author in the present paper deals with the anatomy of its heart.

HISTORICAL RESUME

Gegenbaur (1891) studied the conus arteriosus of Chondrostei, Polypterini and Lepidostei. Senior (1907) Hoyer (1900) and Smith (1918) had discussed the fate of the conus arteriosus in the heart of the teleosts. Parsons (1929) made a comprehensive study of the conus arteriosus of Raia Acanthias Pristiurus, Lepidosteus Polypterus Ceratodus Protopterus, Acipenser Amia, Megalops, Symbranchus Gymnarchus, and Loricaria. Goodrich (1930) says that in typical teleosts the conus however has been practically abolished Awati and Bal (1931) examined the heart of globe fishes Mott (1930) gave a general account of the heart of *Anguilla anguilla* Prakash (1953) studied the heart of *Heteropneustes fossilis* with a special reference to its conducting system. Singh (1960) investigated into the structure of the heart of *Mystus aor Wallago attu, Hilsa ilisha, Labeo rohita, Ophicephalus striatus* and *Mastacembelus armatus*

MATERIAL AND TECHNIQUE

Specimens of *Clarias magur* for the present study were collected from the river Chalakudi and the ponds in the vicinity of Christ College at Irinjalakuda. Besides preparing thick macroscopic sections in the various planes passing through the different regions of the heart of the fishes preserved in 10% formalin serial sections of the heart of the fishes fixed in Bouin s picroformol were cut 8-10 micra thick in transverse, sagittal and frontal planes. These were stained either with Eosin and Delafield s haematoxylin or with Mallory s Triple Stain.

THE HEART

(a) *General account*

The heart is situated immediately posterior to the visceral arches in close proximity with the gills and a little anterior to the insertion of the pectoral fins. The two large and bony cleithra meeting in the mid-ventral line afford sound protection to the heart on its ventral side. The heart

(Fig 1) consisting of a sinus venosus, atrium, ventricle and a bulbus arteriosus, is enclosed within the pericardium.

(*b*) *The Pericardium*

The pericardium (Fig 1) is an extremely thin walled and almost transparent membranous structure. It is conical with the narrow end pointing anteriorly and its wide base extending immediately anterior to the two liver lobes.

(*c*) *The Chambers of the Heart*

1 The Sinus venosus Sinus venosus (Fig 1) the posterior most chamber of the heart is very thin walled. It is transversely extending and its posterior side is abutting the anterior surface of the liver lobes. Opening into the sinus venosus from its posterior side are the two cuvierian ducts and the two hepatic veins and from its antero-dorsal region is the inferior jugular vein. Its spacious lumen is not divided by muscle fibres traversing in a criss-cross manner It communicates with the atrium by the sinu-atrial aperture which is guarded by a pair of sinu-atrial valves. These asymmetrical valves are the extensions of the muscular atrial wall and they extend freely into the lumen of the atrium (Fig 2)

2 The Atrium. Although the configuration of the atrium is conditioned by the quantity of blood present in it three atrial lobes viz., a small anterior lobe and two larger posterior lobes can be made out (Fig 1). The atrium opens into the middle of the ventricle by the atrio-ventricular aperture guarded by a pair of atrio-ventricular valves inserted at the atrial wall with their free tips projecting into the lumen of the ventricle.

3 The Ventricle The ventricle (Fig 1) is a thick cylindrical structure with a slightly concave anterior end and a bluntly pointed posterior end Besides the various microscopic channels among the ventricular muscles two distinct and spacious channels are observed Immediately posterior to the junction of the bulbus arteriosus and the ventricle is the large central channel (lumen) of the ventricle which becomes narrow towards the posterior region. The other channel is disposed in the antero-posterior direction on the right side of the ventricle. In fact the atrium opens into this channel of the ventricle Anteriorly it opens into the central lumen of the ventricle and posteriorly after becoming wider it stops short a little posterior to the atrio-ventricular aperture (Fig 3)

4 The bulbus arteriosus The greyish white coloured bulbus arteriosus (Fig 1) is nearly half the length of the ventricle. Anteriorly it has a very irregular outline enclosing a triangular lumen Its wall is made up of an inner layer of compact muscle-fibres invested by an outer layer of loose fibrous connective tissue. The lumen at the middle of the bulbus arteriosus is square and from the opposing walls two prominent ridges project into the lumen (Fig 4). To each of these ridges a pair of valves are attached. In the posterior end

portion of the bulbus arteriosus each valve is seen meeting its fellow of the opposite side forming a single ribbon like structure (Fig 5) Posteriorly each bulbo-ventricular valve is attached to the posterior wall of the bulbus arteriosus and it descends into the lumen of the ventricle for some distance getting attached to the muscles of the ventricle and then it curves upwards (Fig. 3)

THE WORKING OF THE HEART

The sequence of the working of the various chambers of the heart has been observed in live specimens of *Clarias magur* dissected and kept immersed in 1% saline solution. The two cuvierian ducts the two hepatic veins and the inferior jugular vein convey the blood to the sinus venosus whence the blood enters the atrium through the sinu atrial aperture The blood from the atrum is forced into the ventricle through the atrio-ventricular aperture due to the powerful contraction of the atrium. Powerful contraction of the ventricle results in the flow of the blood into the bulbus arteriosus the ventral aorta and the associated blood vessels.

DISCUSSION

Singh (1960) has noted in *Wallago attu* that one of the sinu-atrial valves is extending forwards into the atrial cavity and adhering to its wall even beyond the atrio-ventricular aperture. Although a pair of sinu-atrial valves are present in *Clarias magur* they simply project into the lumen of the atrium and none of them, however traverses the atrium so as to extend beyond the atrio-ventricular aperture. These valves serve to prevent the reflux of the blood into the sinus venosus from the atrium.

Mitra and Ghosh (1937) in *Clarias magur*, Karandikar and Thakur (1954) in *Sciaenoides brunneus* and Singh (1960) in *Wallago attu* have reported the occurrence of four atrio-ventricular valves—two big and two small valves guarding the atrio-ventricular aperture In *Clarias magur* however only a pair of atrio-ventricular valves are present. These valves do not permit the regurgitation of the blood into the atrum from the ventricle.

Singh (1960) states that the bulbus arteriosus is a non-muscular structure in Siluridae. In *Clarias magur* however the bulbus arteriosus is a highly muscular organ.

Although much of the blood would flow from the bulbus arteriosus into the ventral aorta part of the blood would pass through the V shaped groove at the distal end of the bulbo-ventricular valves and flow down into the space bounded laterally by the wall of the bulbus arteriosus and the longitudinal portion of the bulbo-ventricular valves and posteriorly by the semi-lunar portion of these valves This blood exerts pressure in such a way on each bulbo-ventricular valve that they lie in close contact with each other preventing the reflux of the blood into the ventricle from the bulbus arteriosus

Further the opening and closing of bulbo-ventricular valves are facilitated by alternate contraction and expansion of the ventricular muscles attached to these valves

Summary

1 The bulbus arteriosus is a muscular structure.
2 The ventricle has a definite channel into which the atrium opens
3 The atrium has a small anterior lobe and two larger posterior lobes
4 The sinu atrial valves are asymmetrically disposed.
5 Two hepatic veins open into the sinus venosus.

Acknowledgements

I am extremely thankful to Prof. B C Mahendra for loaning me the necessary literature on the heart of fishes from the Library of the Academy of Zoology and to Rev Fr A. Gabriel, C. M. I., Principal, Christ College, Irinjalakuda for the laboratory facilities I have enjoyed. I am also indebted to Mr K. K. Francis and Mr T Unnikrishnan for procuring me live specimens of *Clarias magur*

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Abbreviations used in the Figures

A —Atrium.
B. A. —Bulbus arteriosus.
I J V —Inferior jugular vein.
I L. —Inner layer of bulbus arteriosus.
L. A. —Lumen of atrium.

L.B.A —Lumen of bulbus arteriosus
L. C. —Longitudinal channel
L. S. V —Lumen of sinus venosus
M.B.V —Ventricular muscles attached to the bulbo-ventricular valves.
O L. —Outer layer of bulbus arteriosus
P —Pericardium.
P F —Pectoral fin.
S.A. V —Sinu-atrial valve.
S. C. —Spacious central channel.
S. V —Sinus venosus.
V —Ventricle.
V A. —Ventral aorta.
V.B.V —Bulbo-ventricular valve.

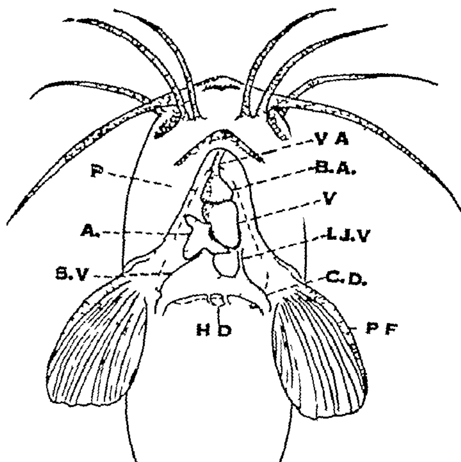


Fig. 1. Dissection of Clarias magur from the ventral side showing the heart. ×2.

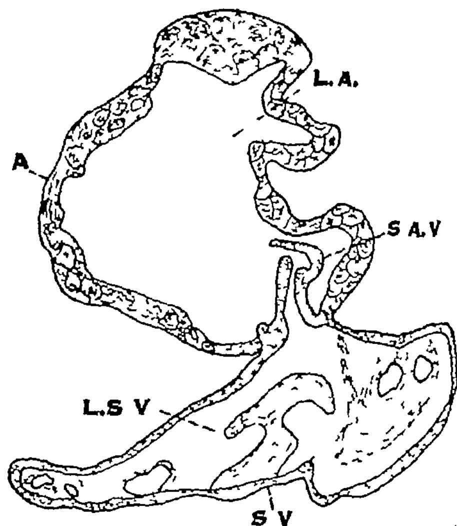


Fig 2 Sagittal section of the trium and the sinus-venosus showing the ... valves. ×80

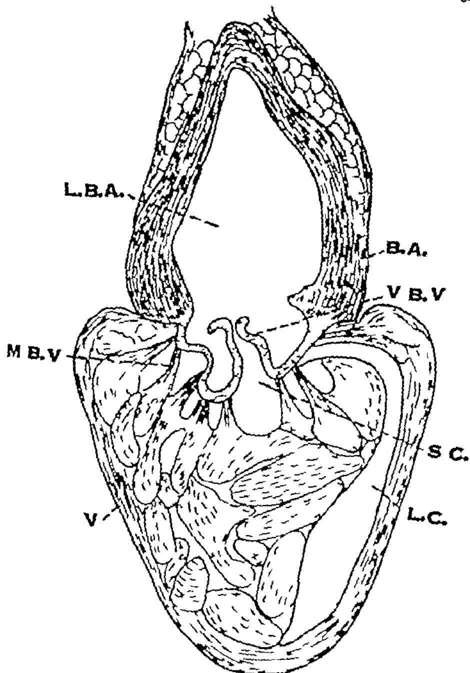


Fig. 3. Sagittal section of the heart showing the blood-channels, the semilunar portions of the bulbo-ventricular valves together with their attached ventricular muscle bundles. × 80

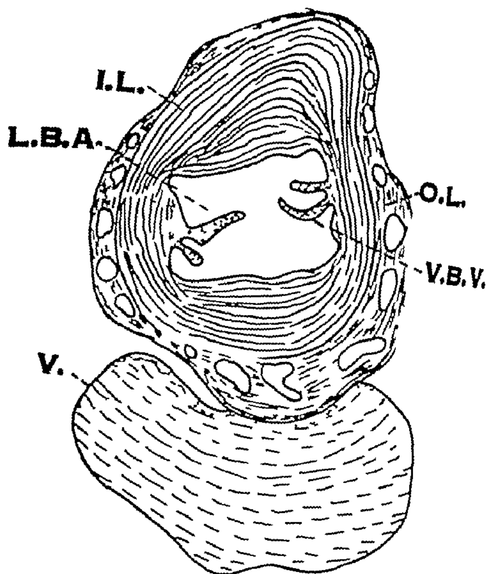


Fig 4 Transverse section through the middle of the bulbus arteriosus. X 60.

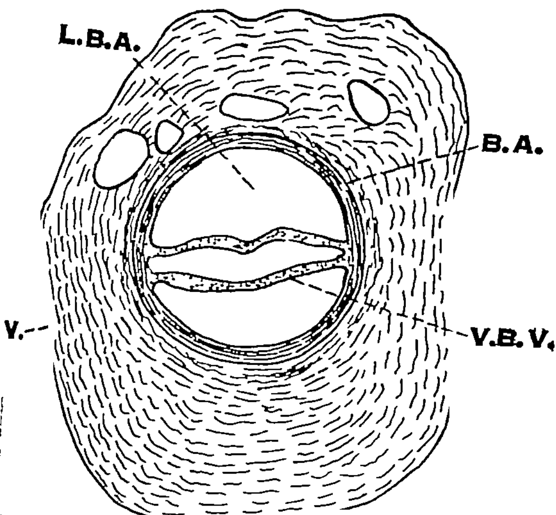


Fig. 5. Transverse section of the heart through the posterior region of the bulbus arteriosus. ×80.

STUDIES ON PATHOLOGY OF CHRONIC RESPIRATORY DISEASE OF POULTRY IN INDIA

I PATHOLOGICAL CHANGES IN FIELD CASES

BALWANT SINGH* AND C M SINGH
Department of Pathology & Bacteriology
U P College of Veterinary Science & Animal Husbandry Mathura India.

The prevalence of chronic respiratory disease (CRD) has only recently been established in India with the work of Pathak and Singh (1959) when they isolated pleuropneumonia like organisms (PPLO) from living and dead birds showing gross lesions similar to CRD Since then the studies conducted in the Department of Pathology and Bacteriology at this Institution during the past few years indicated the wide prevalence of this disease in not only various poultry farms of this State but also in poultry farms of other States.

However the occurrence of CRD in relation to PPLO has long been recognized in other advanced countries particularly U.S.A. Jungherr and Luginbuhl (1952 a, b, c) and Jungherr *et al* (1953) described the lesions of CRD as the fibrino purulent serositis and mucoid sinusitis Microscopically the lesions were lymphofollicular foci in lungs, trachea and air-sacs the multiple granulomas in the lungs and air-sacs and sometimes in the visceral pericardium. Gray (1953) mentioned the presence of lymphofollicular lesions in trachea and air-sac mucosa, grossly seen as beading reaction. Jungherr *et al* (1955) examined 70 grossly positive and 130 grossly negative cases histopathologically They demonstrated 42 5 % lesions in trachea and 11 7% lesions in lungs in grossly negative cases thus stressed the desirability of carrying out histopathological examination in PPLO infection Fahey and Crawley (1954) stated that the disease produced by PPLO and CRD virus was more severe than that produced only by PPLO Many authors produced lymphofollicular lesions in lungs trachea and air-sacs by inoculation of PPLO alone (Johnson 1954 Olesiuk and Van Roekel 1960 and Jungherr 1960)

The present studies were undertaken to elucidate the incidence and distribution of pathological lesions in this syndrome with particular reference to histopathological examination.

MATERIAL AND METHOD

The tissues for this study were collected from the carcasses received from Government Poultry Farm, Mathura and from some private breeders and autopsied in the Department of Pathology and Bacteriology and materials

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* Present address Asst. Prof. of Pathology College of Veterinary Science U.P Agricultural University Pantnagar (Nainí Tal)

obtained from other sources like Tarai State Poultry Farm, Nagla Poultry Farm, Hessarghatta D I O Bhopal and Superintendent, B. P Section, Hyderabad (A.P) The gross lesions if any were noted. The gross pathological examinations were conducted on 2943 birds under three months and 538 birds over three months of age. The histopathological examination was carried out on lungs, trachea and air-sacs of 419 cases above three months and 433 cases below three months of age.

The tissues were also collected from 610 embryos which died on 18-21 days of incubation during hatching at Government Poultry Farm, Mathura and also from 60 dead in shell embryos in experimental hatching in this department from PPLO free flock. The tissues from 10-20 embryos were preserved and processed in one lot.

The sections of air-sac, lungs, heart and liver were cut 5 microns, trachea 6 microns. All the sections were stained by routine haematoxylin and eosin. The Maccalum Good Pasture (Krajian and Gradwohl 1952) and Periodic Acid Schiff stain were also done for the demonstration of bacteria and fungus materials.

RESULT

On the basis of pathological examination the cases were grouped into the following categories

1 *Cases showing both gross and histopathological lesions resembling CRD*

The gross lesions resembling CRD and characterized by consolidation of lateral borders of the lungs congestion of trachea and lungs and thickening of air-sacs usually with a deposition of caseous exudate in their cavities were observed in 26 birds over three months of age (Fig 1) two of which also revealed perihepatitis and pericarditis.

The histopathological examination revealed lymphofollicular foci in the tertiary bronchi of lungs in two cases and giant cell granulomas in lungs of 21 cases. One of these two cases having lymphofollicular foci in the lungs also revealed similar foci in trachea and air-sacs alongwith early stage of granuloma formation and fully formed giant cell granuloma in the lungs (Figs. 2, 3 4 5) The other case besides showing lymphofollicular foci in air sac also revealed giant cell granuloma in lungs, tuboalveolar elongation and vacuolar degeneration in the tracheal mucosa. Lymphofollicular foci in the submucosa of one trachea, vacuolar degeneration and tuboalveolar elongation of intra-epithelial glands of tracheal mucosa in 22 cases and diffuse lymphocytic infiltration in tracheal submucosa of 24 cases were observed. Sero-fibrinous exudate with infiltration of macrophages and heterophils and lymphoid follicles was observed in the air-sacs of 23 cases and lymphoid follicles only in six cases Twentytwo cases out of 26 revealed lesions in all the three organs *i. e* lungs, trachea and air sacs, one case showed lesions only in

trachea and three cases had lesions in trachea and air-sacs. One case showed suppuration in the lung and two cases showed diffuse heterophilic and macrophagic infiltration in the lung parenchyma with diffuse mononuclear infiltration in th tracheal submucosa and air-sac. Isolation of PPLO was attempted in 13 and only eleven yielded PPLO

2. *Cases apparently showing no gross lesions but having microscopic lesions*

The histopathological examination of 826 cases without any gross lesions was conducted. These cases include 433 cases under 3 months of age and rest of the 393 cases were above 3 months of age. The histopathological lesions were observed in 50 out of 393 cases

Nine cases revealed lymphofollicular foci and twelve cases showed giant cell granuloma in the tertiary bronchi of lungs One of these cases having lymphofollicular lesion in lung also revealed giant cell granuloma in the same section. Lymphofollicular foci in the tracheal submucosa of 24 cases vacuolar degeneration and tuboalveolar elongation of intraepithelial glands of tracheal mucosa in 33 cases and diffuse mononuclear infiltration in the submucosa of trachea in 33 cases were observed. Air sac showed serofibrinous exudate with macrophagic and lymphocytic infilteration only in one case and lymphofollicular focus in another case. Lungs and trachea were involved only in twelve cases. Cultures were taken from 22 cases from which only 13 yielded PPLO.

Two out of 433 cases under three months were noticed to have microscopic lesions, one case was having giant cell granuloma which was of mycotic origin as shown by P A. S stain and the other showed lymphoid follicle in the tracheal submucosa.

3. *Dead-in-shell embryos*

The histopathological examination of 240 dead-in-shell embryos from Poultry Farm, Mathura revealed perivascular macrophagic and heterophilic infiltration in 58 cases but similar lesions were also noticed in 5 out of 60 dead-in-shells from PPLO free layers indicating the insignificance of these lesions.

Discussion

There has been considerable confusion in the literature about the pathology of CRD as a definite disease entity vis-a-vis CRD complex or mixed infection although several workers have tried to differentiate the lesions caused by PPLO alone and PPLO in combination with other factors.

In the present investigation 26 cases showed gross lesions of CRD which are considered to have been complicated with secondary infections as similar findings have been reported by many authors (Van Roekel and Olesiuk 1953, Fahey and Crawley 1955 b, Olesiuk and Van Roekel 1960) These views are further strengthened by the report of Bankowski (1961) who also described a

number of agents along with *Mycoplasma gallisepticum* as the cause of air-sac infection and CRD as a specific disease entity caused by *Mycoplasma gallisepticum* Pericarditis and perihepatitis were observed in two out of 26 above mentioned cases which are in accordance with the findings of Van Roekel and Olesiuk (1952) Tudor (1953) and Gray (1933) who described such lesions in mixed infections.

Histopathologically the lymphofollicular lesions in the lungs of two cases trachea of two cases and in the air-sacs of six cases were observed. These lesions are similar to those described by Jungherr and Luginbuhl (1952) Gray (1953) Van Roekel and Olesiuk (1953) Jungherr *et al* (1953), Jungherr (1960) and Adler (1960) who stated the lymphofollicular foci as the characteristic lesions of CRD However the lymphoid follicles in the air-sac has also been produced by Johnson and Domermuth (1956) by inoculation of Fahey and Crawley virus alone and Beasley *et al* (1961) also reported these lesions by Ornithosis agent in turkeys free from PPLO and Ornithosis infection. On the contrary similar lymphofollicular lesions are reported to have been produced in air-sacs of 4 week old germ free turkeys by PPLO alone (Sniebert *et al* (1959) Similar lesions were also observed in lungs of nine cases, trachea of 25 cases and air-sac of one case among the 52 cases having only microscopical lesions without any gross indication of the disease

Two of the 26 cases showed complicated lesions. One of these cases showed lymphoid follicle in the tertiary bronchi of lungs, air-sac and trachea, the so-called pathognomonic lesions. In addition to these, lungs revealed early stage of granuloma formation and fully formed giant cell-granuloma diffuse lymphocytic infiltration in the trachea and serofibrinous exudate in the air-sac were also seen The other case presented lymphoid follicles in the tertiary bronchi of lungs, and in air sac and tubuloalveolar elongation and vacuolar degeneration of tracheal mucosa with serofibrinous exudate in the air-sac which clearly showed the complication of the basic lesion of PPLO by other secondary agents Similarly another case without gross lesions also revealed lymphoid follicle in the tertiary bronchi and giant cell granuloma in the lungs.

Twentyone of the 26 cases revealed gaint cell granuloma in the lungs mostly in the tertiary bronchi characterized by formation of multinucleated giant cells surrounding the cellular debris alongwith lesions in trachea and air sac. Similar findings are reported by Jungherr *et al* (1953), Van Roekel *et al* (1957) who described giant cell granuloma as the characteristic lesion of CRD The granulomatous lesions were also noticed in the lungs of 13 cases among 52 cases with no gross lesion One of these 13 showed mycotic infection by P. A. S stain However Gross (1957) claimed to have produced similar lesions with *E. coli* alone and Beasley *et al* (1961) also produced granulomatous lesions in lungs of turkeys by Ornithosis agent alone So these lesions are of little significance in the diagnosis PPLO infection except after exclusion of other

possibilities of other agents capable of causing such lesions by detailed cultural and histopathological studies.

The lesions consisting of tuboalveolar elongation and vacuolar degeneration in intraepithelial glands of tracheal mucosa and diffuse mononuclear infiltration in the submucosa serofibrinous exudate with cellular infiltration in the air-sac can only be considered as suggestive lesions of CRD in accordance with the findings of Jungherr *et al* (1953) Gross (1957) Adler and Shrifrine (1960) and Jungherr (1960)

One of the 26 cases with gross lesions showed suppuration in lungs and two cases revealed diffuse macrophagic and heterophilic infiltration in lungs with associated suggestive lesions in air sac and trachea. These cases cannot be ascribed to PPLO infection similar to those described by Jungherr *et al* (1955) who failed to detect microscopic lesions in 3 out of 12 cases having gross lesions So it is clear from above studies that the histopathological examination is of great importance to establish the lesion of PPLO as 50 of 593 (12 7%) cases without gross lesions revealed only histopathological lesions similar to the findings of Jungherr *et al* (1955) who demonstrated only microscopic lesions in 42·5% cases in trachea and 11 7% in lungs in 130 grossly negative cases.

SUMMARY

In the present studies 2943 birds under 3 months of age and 558 over three months were examined grossly in routine post-mortem examination. Only 26 cases out of 558 over 3 months were noticed to have gross lesions.

Twentythree out of 26 cases with gross lesions showed microscopic lesions resembling to those caused by complicating agents in addition to PPLO Fifty two out of 826 cases without any gross lesions revealed histopathological lesions varying from lymphofollicular lesions to giant cell granuloma in the lung with associated lesions in trachea and air-sacs.

The present study stresses the importance of carrying out histopathological examination for the diagnosis of CRD in addition to gross examination.

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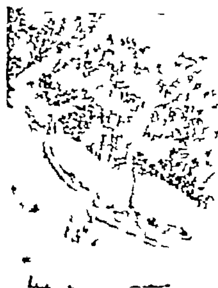
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Fig 1 2220P/61 Showing the presence of caseous xudate in thoracic and bdominal air sacs and thickened pericardium



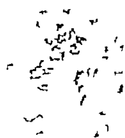
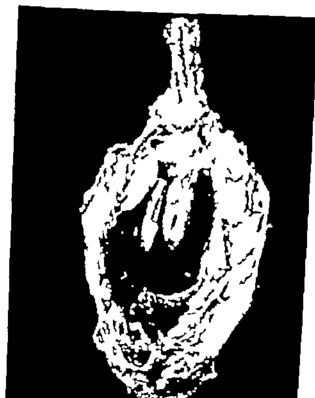


Fig. 2 1458P/61 Trachea showing l nal h l cells l ongation of intra-epithelial glands. H

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[illegible] 1 2220P/61 Showing [illegible]nce of caseous exudate in [illegible] and abdominal air sacs [illegible]lved pericardium



Fig. 2 1450P/61 Trachea showing lymphoid follicles and tubo-alveolar elongation of intra-epithelial glands. H. & E. 180.

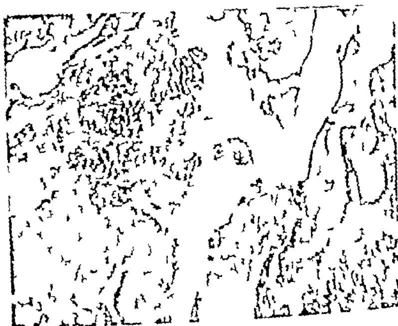


Fig 3 1458P 61 Lung showing the lymphofollicular focus in the tertiary bronchi along with light serous exudate in the lumen. H & E×180



Fig 4 [illegible] showing giant cells in the lumen of tertiary bronchi and [illegible] and [illegible] vascular wall which may be the early stage of [illegible] [illegible] H & E ×180

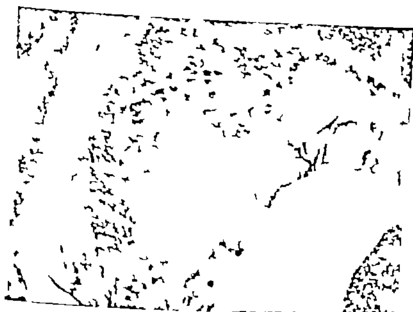


Fig. 3. 1456P/61 Lung showing the giant cell granuloma necrotic cellular mass surrounded by multinucleated giant cells. H. & E.×180

A NOTE ON THE INCIDENCE AND FLOWER COLOUR PREFERENCE IN *SYRPHUS BALTEATUS* (DE GEER) (SYRPHIDAE DIPTERA)*

J L NAYAR

Senior Research Fellow C S I R. Ministry of Education,
School of Entomology St John s College Agra

This paper records the occurrence and colour preference of different flowers by *Syrphus balteatus* (De Geer) in Agra during 1961 This fly is one of the widely distributed *Syrphus* and is known to be extremely common in the East, all over Europe, Madeira Canaries, N Africa and Asia including Japan. Brunetti (1923)† records that these flies are found during whole of the warm weather whereas its occurrence is mainly confined to the spring months and hardly a specimen is available after spring

In the outer ranges of Himalaya (Narkanda, 9,600 ft. 40 miles away from Simla on the Hindustan Tibet Road) this species was also collected during the autumn months but was rather scarce.

Collection Method—For the study of incidence the flies were collected regularly for a fixed period between 9·00 a. m. to 11 30 a m. Collection by the sweep net was found unsatisfactory Being swift fliers they always escaped at the slightest disturbance. Therefore they were collected while resting A small glass tube (3 × 1") was used to trap the fly when sitting on the flowers or the foliage. The mouth of the tube was placed from the posterior side of the fly as soon as it came to rest on the flower or the leaf It suddenly flew towards the blind end of the tube and simultaneously the mouth was corked. Though the procedure was ted ous yet it was found to be rewarding

The daily collection was sorted out and analysed on the basis of plant species from where the flies were collected.

Habits—The adults are diurnal, pollen grain eaters and occur in parks and gardens, visiting flowers and the leaves. Before feeding, the fly comes in the vicinity of a flower and hovers over its top standing a little above it and at last clings to the flower head by its legs and starts taking the pollen grains The feeding lasts for a few seconds only and soon the fly lots another flower either in the same bed or elsewhere. The flies which were kept in a small cage and fed over pollen grains survived for a day only probably because their flight was restricted When feedi g the wi gs remain stretched outwards and when the flies are at rest on th leaf surface the wings lie folded over the abdomen. During flight the wings are hardly

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* Contribution No. 97 from th School of Entomology St. John's College Agra.

† Brunetti, R. 1923. *Faun. Brit India* Diptera 3 82-84

visible on account of their rapid beating The flies are heliotropic and disappear soon after sun-set.

General observations—*Syrphus balteatus* (De Geer) makes its appearance in Agra only in the last week of February Its population gradually increases and touches the peak somewhere in the middle of March and again shows a very marked fall till the end of April, after which it disappears. It feeds on the pollen grains of various plants belonging to the families Labiatae Scrophulariaceae, Solanaceae Compositae, Verbenaceae Umbelliferae, Papilionatae, Chenopodiaceae Ranunculaceae, Caryophyllaceae, Polemoniaceae and Rutaceae.

TABLE 1

Statement showing the number of flies caught weekly the range of maximum temperature and their weekly and monthly percentages out of total collection in 1961

Month	No. of flies	Range of max. temp.	Weekly percentage out of total catch.	Monthly percentage out of total catch
1 February				
4th week	3	25–27°C	0·4	0·4
2 March				
1st week	82	31–37°C	11·0	61·8
2nd week	78	32–34°C	10·4	
3rd week	175	31–35°C	23·5	
4th week	125	29–35°C	16·8	
3 April				
1st week	127	31–37°C	17·2	37·7
2nd week	78	33–36°C	10·4	
3rd week	56	34–45°C	7·5	
4th week	20	36–44°C	2·7	
Total number of flies collected	744			—

This data show that the maximum number of flies available was during March representing 61·8% of the whole collection In April the percentage came down to 37·7 (Table 1 Fig 1) After April the flies were not found.

In the last week of February only 0·4% of the whole collection were collected The highest weekly percentage of the catch was recorded from the third week of March (23·5%) A month later in the third week of

April the percentage decreased to 7.5. The last week of April revealed only 2·7% of the total catch.

Further this study (Table 2 and fig 2) has also exhibited a well pronounced preference for white flowers About 69·9 % flies were collected from white flowers of *Iberis* sp Besides 6.8% were collected from other white flowers namely *Raphanus sativus Petunia alba Foeniculum* sp *Phlox* sp. and *Brachycome* sp. This means more than 76% of the total catch came from the white flowers. Yellow flowers of *Glycine soja* came second in preference and attracted 11.5% of the total catch The attraction by other yellow flowers (*Brachycome* sp., *Eschscholtzia* sp. and *Sonchus* sp.) was extremely low representing only 0·9% The blue flowers of *Ocimum basilicum*, *Salvia* sp and *Brachycome* sp yielded 8.7% of total catch and the red flowers of *Papaver* sp., *Antirrhinum* sp. and *Saponaria* sp were the least preferred with only 1.7% catch These observations revealed that *Syrphus balteatus* (De Geer) is predominantly attracted by the white flowers and the yellow blue and red flowers fall in the successive order of preference.

During February all the flies were collected from *Iberis* sp having white flowers. In March about 87% flies came from white flowers. In the 1st, 2nd, 3rd and 4th weeks of March the white colour attracted 87.7% 88·4% 87·9% and 86·4% of the flies respectively

In April the catch from the white flowers was 59·8% A considerable rise was noticed from the yellow flowers, indicating 22.5% as compared to 3.8% in March. The blue colour also showed a significant rise to 15.8% of the total catch.

It has been found that the range of temperature has profound effect on the preponderance of these flies During the last week of February with a range of maximum temperature of 25 27° C the seasonal catch was hardly 0·4% As the temperature rose more and more flies emerged. In the third week of March with 31 35°C range of temperature 23·5% flies were captured. In the 4th week of March and 1st week of April with the range of maximum temperature between 29° 37°C about 17% flies per week were captured. Here after the population of flies decreased considerably on the onset of hot season.

It is a well known fact that the *Syrphus* flies are attracted to heavily aphid infested plants for egg laying to ensure sufficient food supply for the young larvae, which feed on aphids. In the present investigations, however it has been found that the plants under observations except *Raphanus sativus* were not at all infested by aphids yet *Syrphus balteatus* exhibited a pronounced tendency to visit white flowers for feeding It is worthwhile to record her that during the year 1961 the area under investigation was devoid of any aphid infestation Normally these aphids apart from providing food for future *Syrphus* larvae also provide honey dew to the adults. But as already

pointed out *Syrphus balteatus* in the absence of aphids visited white flowers more frequently than any other indicating a clear preference for colour

SUMMARY

Syrphus balteatus (De Geer) occurs from the last week of February to the end of April. The fly is diurnal pollen eater showing marked preference for white flowers The yellow blue and red flowers fall successively in order of preference by the fly The optimum temperature suitable for the breeding of the fly ranges between 30°-35° C

ACKNOWLEDGEMENTS

My sincerest thanks are due to Dr T Singh, Professor of Zoology and Entomology School of Entomology St John s College, Agra for a valuable guidance and facilities for work. I am highly indebted to Dr Santokh Singh of School of Entomology for his kind suggestions and help. My thanks are also due to Shri D P Gupta of the Botany Department for identification of the plants

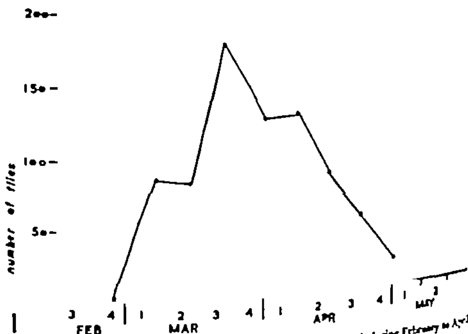


Fig 1 Graph showing the availability of *Syrphus balteatus* (De Geer) during February to April

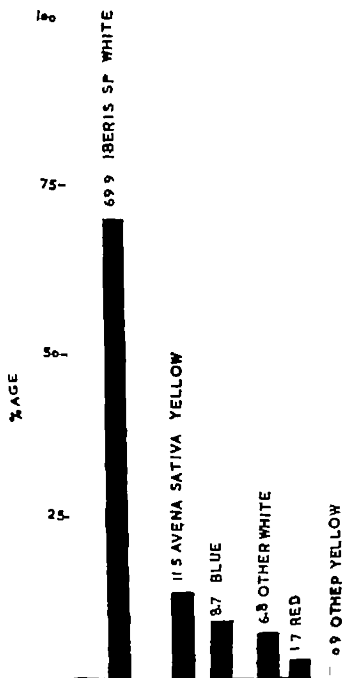


Fig. 2. Histogram showing the flower colour preference by Syrphus balteatus (De Geer)

SHARPNESS OF THE COLLOID ELECTROLYTE BOUNDARY AS A CRITERIAN TO MEASURE THE MOBILITY OF COLLOIDAL MICELLE. III ELECTROPHORESIS OF AS_2S_3 SOL.

P D BHATNAGAR[1] AND ABANI K. BHATTACHARYA[2]

The importance of choosing a suitable electrolyte as supernatant liquid to produce a sharp descending boundary was discussed for positive sols of $Cr(OH)_3$ and $Fe(OH)_3$. On the basis of Kohlrausch Weber theory for ionic boundaries it was reasoned out that the more probable value of the electrophoretic mobility of the colloidal micelle would be obtained when the colloid micelles are allowed to behave as leading ons by properly choosing a suitable electrolyte to produce a sharp descending boundary.

The present paper deals with our studies with colloid-electrolyte boundary of the negatively charged AS_2S_3 sol with various equiconducting electrolytes as supernatant liquids. The colloid electrolyte interaction reported with positive sols has been observed to play a distinct role in these cases.

The conclusions drawn in the earlier communication regarding the limitation of the analogy of the colloid-electrolyte boundary and pure ionic boundaries, and the greater significance of a sharp descending boundary to give more representative values for the migration velocity of colloidal micelles, have been supported from the observations of the negatively charged AS_2S_3 sol.

EXPERIMENTAL

The apparatus consists of two parts after Tiselius pattern the electrode vessel and the main U tube fitted by standard glass joints at P_1 and P_2. M_1 M_2 are the electrodes supplying a constant current the device for maintaining a constant current is described below —

A sharp-cutoff pentode 6 S J 7 whose plate current is independent of its plate voltage within a range of 40 to 500 volts (R C. A. Receiving tube manual P 0·211) is used in series with the U-tube as shown in the circuit (Fig 1)

The voltage drop across the whole U tube is first of all determined by the potentio-V T V M. system flowing the required current (0·1 to 0·4 milliamps) to flow through the circuit. The supply voltage from the rectifier is so adjusted that the plate voltage remains sufficiently above 40 volts. Now

Present address 1 Reader in Chemistry Regional College of Education Ajmer
2. Head of Chem. Deptt. J & K. University Srinagar Kashmir

because of the characteristics of the tube 6 S J 7 any change in the voltage drop across the U-tube due to changes of the resistance will not change the current in the circuit as long as the plate voltage remains between 40 to 500 volts. The grid of the valve is connected to a series of batteries to give it the required negative potential for the desired amount of current (0·1 to 0·4 milliamps)

Observations and Discussion

According to the theoretical considerations of Kohlrausch and Weber for ionic boundary a stable boundary can be realised when the slower moving ion follows the faster moving ion such that both move with equal speed An extension of such considerations has been put to practical tests for ionic boundaries and many more suggestions have been introduced by eminent workers in this field

J N Mukherjee[1] (1928) after a large number of electrophoretic measurements was first to conclude the necessity of securing an identical ionic environment during the movement of the colloid particles. As the use of ultrafiltrate as the upper liquid is not free from objection, he suggested equiconducting supernatant layer of a suitable electrolyte

Henry and Brittain[2] (1933) on basis of mathematical treatment inferred that "the use of falling boundary shall be undoubtedly preferable to that of the rising"

Considering the conclusions of both these pioneers a suitable electrolyte was chosen as supernatant liquid to (1) produce an identical ionic environment during the motion of the colloid particles, and (2) to produce a sharp descending boundary As the counter ions in case of As_2S_3 sol are H^+ ions equiconducting HCl H_2SO_4 H_3PO_4 Oxalic acid and acetic acid were tried as the supernatant layer at the boundary

Plate nos 1 and 2 show that a sharp ascending and a diffuse descending boundary is obtained with equiconducting HCl, H_2SO_4 H_3PO_4 and oxalic acid and it was only with acetic acid that a sharp descending boundary was obtained It will be seen from the plate nos 1 and 2 that the inference of the Kohlrausch Weber[3] theory can only be qualitatively applied to interpret the sharpness of colloid-electrolyte boundaries

The descending boundary gets diffused when equiconducting HCl H_2SO_4 H_3PO_4 oxalic acid are used as supernatant liquids because here the mobility of the colloid micelles acting as leading ions is lesser than that of Cl^- SO_4^{--} PO_4^{---} and oxalate ion which behave here as indicator ions (table no 1) While with acetate ion as indicator ion (whose mobility is lesser than that of the colloid micelles) the boundary acquires a desired sharpness Similar is the case of ascending boundaries which are sharp

when the Cl^- SO_4^{--} PO_4^{---} Ions (here acting as leading ions) are followed by colloid micelles of lower mobility (behaving as indicator)

The case of sharp ascending boundary with acetic acid is however not in accordance with the expectations of the Kohlrausch theory Also it will be seen for table no. 1 that the colloid micelles do not appear to move with the velocity of the leading ions as required by the theoretical treatment of Kohlrausch and Weber The possible colloid-electrolyte interaction as discussed in our previous communication seems to be a likely cause of such deviations, which is indicated by the variations of the potential at the subsidiary electrodes during the electrophoretic movement under constant current (Fig 2) According to Ohms law for constant current between the subsidiary electrodes, the voltage across them should be constant provided that the resistance of the system remains constant for

$$I_{const} \times R_{const} = V_{const}$$

but as it is an established observation that variations in the voltage across the subsidiary electrodes do take place, it can be plausibly assumed that the resistance changes on passing the current as

$$V_1 = I_{const} \times R_1$$

$$\text{and } V_2 = I_{const} \times R_2 \text{ etc.}$$

Since in our experiments the products of electrolysis were cut off from the main U-tube by the Tiselius arrangement and the effects of polarisation were excluded by using electronic constant current supply at the main electrodes and potentio-V T V M. was used as potential measuring device across the subsidiary electrodes any changes in potential across the main U-tube will be due to the changes in the resistance of the colloid and electrolyte forming the boundary From the studies on the variations of potential with various equiconducting electrolytes as supernatant liquids it has been observed that such variations show different ascending and descending characteristics, (curve no 1 2 3 4) This may be taken as an index that different electrolytes interact with the sol boundary in different ways. If there was no interaction we would expect that, ceteris paribus the curves should be linear and parallel to the time axis in the plot of voltage V/S tim and because this is not so, the colloid-electrolyte interaction seems to be mainly responsible.

To obtain more information about the colloid-electrolyte boundary under the influence of electric field work is in progress

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TABLE 1

Equiconducting supernatant liquid	Ascending boundary				Descending boundary			
	Acetic Acid	H_3PO_4	H_2SO_4	HNO_3	Acetic Acid	H_3PO_4	H_2SO_4	HNO_3
As_2S_3 sol boundary	diffuse	Sharp	Sharp	Sharp	Sharp	Not Sharp	Not Sharp	Not Sharp
Plat no.	2	1	1	1	2	1	1	1
Mobility of anions cms./sec./unit pot. grad.	42	–	79	65	42	–	79	65
Acting as	Leading Ion	Leading Ion	Leading Ion	Leading Ion	Indicator Ion	Indicator Ion	Indicator Ion	Indicator Ion

Temp. 27°C

Mobility of colloid micelle from sharp falling boundary 35 cms /sec./unit pot. grad.

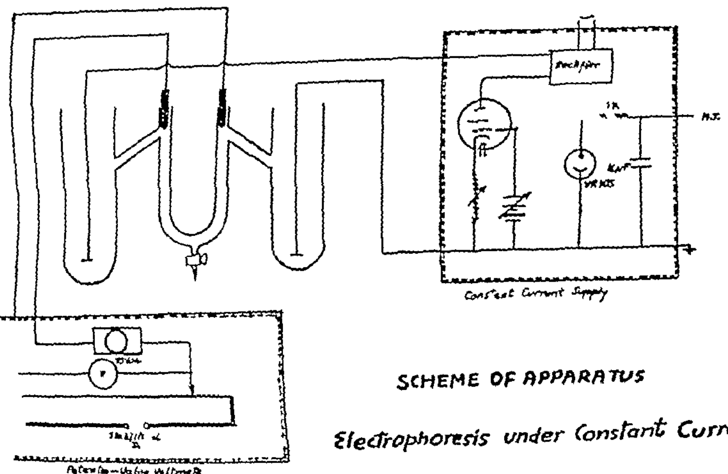
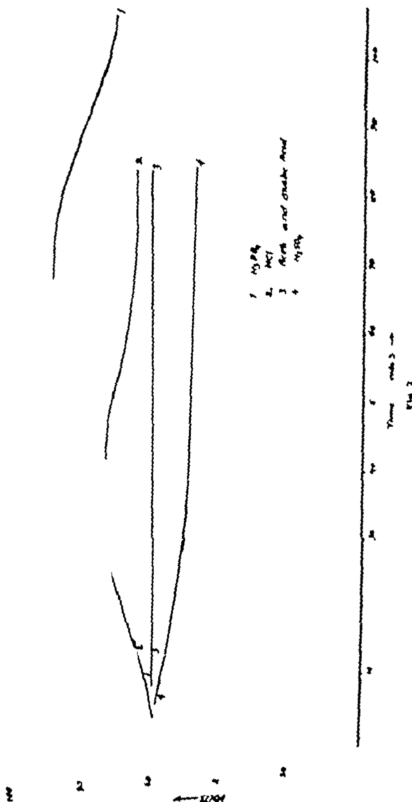

Rectifier
Constant Current Supply
SCHEME OF APPARATUS
Electrophoresis under Constant Current

Fig. 1



As2S3 Sol
Variation of potential across the main U Tube
with various equiconducting supernatant liquids
1 H3PO4
2 HCl
3 Acetic and oxalic acid
4 H2SO4
Time mts →
Fig 2

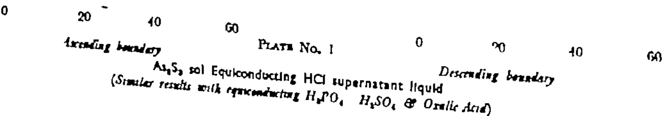
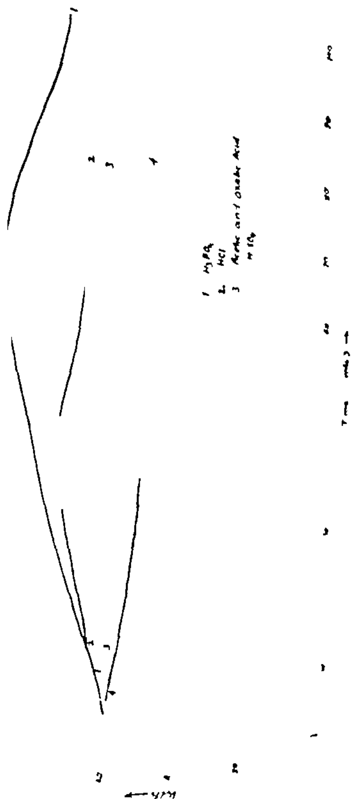


PLATE No. 1

As_2S_3 sol Equiconducting HCl supernatant liquid

(Similar results with equiconducting H_3PO_4, H_2SO_4 & Oxalic Acid)


As_2S_3 Sol
Variation of potential across the main U Tube
with various equi conducting supernatant liquids

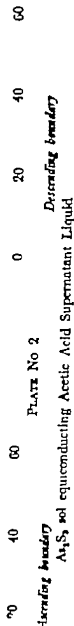


PLATE No 2

As_2S_3 sol equiconducting Acetic Acid Supernatant Liquid

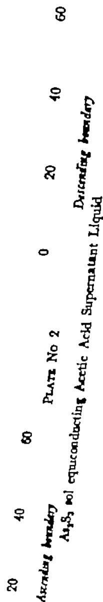


PLATE No 2

As_2S_3 sol equiconducting Acetic Acid Supernatant Liquid



Fig. 1 L. S. of a vegetative bud with conical crown × 175
(a.c.=apical crown, t=thorn primordium).



Fig. 2. L. S. of differentiated bud showing sepal primordia × 175.
(sp.=sepal primordium, .c.=regular apical curve.

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Fig. 1 L. S. of a vegetative bud with conical crown × 175

(a.c.=apical crown, t=thorn primordium).



Fig. 2. L. S. of a differentiated bud showing sepal primordia × 175.

sp.=sepal primordium, .c.=regular apical curve.

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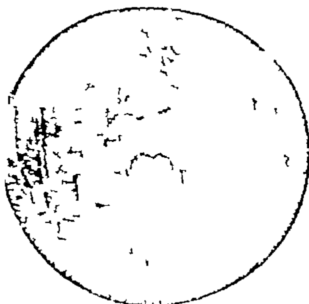


Fig 5 Showing ompletely d loped flower ×17
St.=Stamen G =Gynoeci m p.=petal sp =sepal

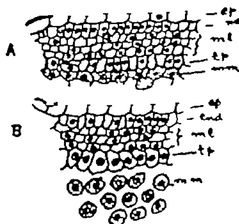


Fig 6 T S. of microsporangium
(A) Compact mass of microspore m l
(B) Showing separation and ... of =end
ep =epidermis,
ml.=middle lay rs, tp.=ta
mm.=microspore mother l

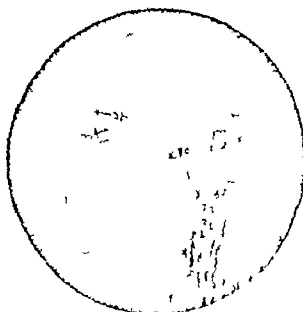


Fig. 3. Showing emergence of petal primordium ×175

pp. = petal primordium.
fc = flattened crown.

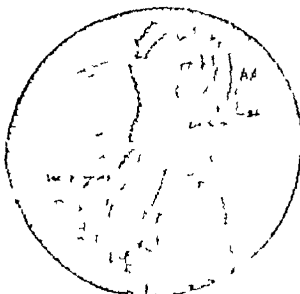


Fig. 4 [illegible] initiation of stamen and carpel pr[illegible]dia ×17
st. p. = stamen primordi[illegible]
[illegible] = carpel primordium
p. pr. = petal primordium
s. p. = sepal primordium
[illegible] = secondary [illegible]



Fig. 5 Showing completely developed flower ×175.
St.=Stamen, Gy =Gynoecium p.=petal sp.=sepal.

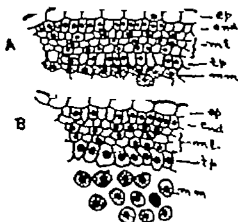


Fig. 6 T. S. of microsporangium
(A) Compact mass of microspore mother cells.
(B) Showing separation and loose mother cells ×400
ep.=epidermis, end.=endothecium,
ml.=middle layers, tp.=tapetum
mm.=microspore mother cell.

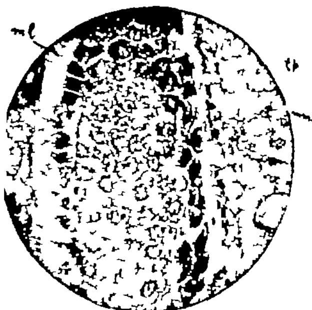


Fig 7 Showing degenerating tapetal layer and degenerated middle layers.
ml.=middle layers. ×770.



Fig 8 Showing tetrahedral arrangement of microspore ×770.
tp.=tapetal layer
tt.=tetrahedral arrangement of microspore tetrads.

STUDIES ON THE MORPHOLOGY AND PHYSIOLOGY OF THE ALIMENTARY CANAL OF *MASTACEMBELUS PANCALUS* (HAM)

V P AGRAWAL AND A P TYAGI
D A V College Muzaffarnagar

INTRODUCTION

The fish *Mastacembelus pancalus* locally known as "Chhota Bam" is abundantly found in Kalinad and other streams of the locality It is an elongated eel-like fish which usually burrows under the soft substratum It can also be kept in the aquarium under the artificial conditions for a considerable period.

It has been considered worthwhile to study the morphology and physiology of the alimentary canal of this burrowing fish which is peculiarly modified in shape in response to this new habit

HISTORICAL

The morphology and histology of the alimentary canal in teleostean fishes have been studied by many workers Rogick (1931) described the digestive tract of minnow *Campostoma anomalum*, Sarbahi (1939) that of *Labeo rohita* while Curry (1939) discussed the digestive tract of *Cyprinus* Al Hussaini (1946 1947 & 1949) has discussed in a comparative way the alimentary canal of a few teleosts in order to correlate the different aspects involved such as feeding habits structure of alimentary canal and physiology of digestion.

The pyloric caeca in various families of Teleostei have been studied by Rahimullah (1941a 1943 & 1945)

Investigations on the physiology of digestion in fishes have been few workers on the food of fishes have made only incidental observations on the physiology of digestion. Rahimullah (1945) instituted a comparison between the physiology of digestion of pyloric caeca in the carnivorous fish *Ophiocephalus striatus* and the herbivorous fish *Osphronemus goramy*

MATERIAL AND METHODS

The specimens of *Mastacembelus pancalus* were collected from K Band which runs within the outskirts of Muzaffarnagar They were kept in an aquarium which was provided with a sandy bottom

To determine the hydrogen ion-concentration of the normal fishes freshly collected specimens were dissected alive The different

of the alimentary canal were carefully separated and the pH in them determined both by capillary indicator and indicator paper methods

For the qualitative analysis of the digestive enzymes in the different parts of the gut and the digestive glands the extracts of the different parts were prepared as follows

About 20 fishes were starved for a week to clear off the gut from any food contents these were then dissected in living condition and different parts of the digestive system were separately ground up with a little thymol and a few drops of glycerol to form a thick uniform emulsion This was diluted with 50% glycerine to make a solution of about 10% strength. The solution after being centrifuged at 3000 r.p.m was kept over toluene for about a week before it was tested for the different enzymes

The Alimentary Canal

Mastacembelus is a carnivorous fish it is a bottom feeder and usually feeds on such minute animals as white worms blood worms, glass worms and such other crustaceans as *Daphnia*

The organs concerned with the capture of food, its digestion and absorption comprise the alimentary canal proper with its associated glands.

The alimentary canal of *Mastacembelus* is a comparatively short tube in a fish about 12 cm. long the total length of the gut being only 5 cm. It forms an almost straight tube opening posteriorly by a small slit-like ventral anus

The alimentary canal of *Mastacembelus* consists of

(a) The mouth and buccal cavity
(b) The oesophagus
(c) The stomach
(d) The intestine and
(e) The rectum

The mouth of *Mastacembelus* is a very small (about 0·4 cm in diameter) terminal opening situated on the narrow long snout-like prolongation of the head The upper lip is extended forward into a trilobed flap which is a soft membranous and mobile structure its distal margin is richly supplied with taste buds and thus serves for the selection of the food from the substratum where the fish burrows. This flap consists of a median stiff solid and pointed process and two lateral soft hollow and blunt projections.

The buccal cavity is a long dorso-ventrally flattened spacious cavity being about 1 5 cm. long and 0·5 cm. wide. The roof of the cavity is formed by the base of the cranium while the sides and the floor are supported

branchial arches and the median urohyal respectively The tongue of the fish is a triangular structure consisting of the glossohyal enclosed in a thick mucous membrane.

The dentition on the roof of the buccal cavity is represented only by the maxillary teeth (palatine and vomerine teeth being absent) which are arranged in a double row on the outer margin of the upper jaw These teeth are directed backwards and are borne on the two premaxillae.

The mandibular teeth on th floor of the buccal cavity are small and pointed they are arranged in double rows on the dentaries of lower jaw

The posterior part of the buccal cavity broadens into the pharynx, about 1·9 cm. long and 0·5 cm wide the pharynx is divisible into a narrow anterior portion and the broader posterior portion. The roof of the anterior part of the pharynx is supported by the base of the cranium while the floor is formed by the hypo-branchial and the median basi branchial elements. It is perforated on the latero ventral margins by the gill slits through which the pharynx communicates with the branchial chamber

The pharyngeal teeth are small and close set, they are d rected back ward and are arranged on a pair of oval patches situated on the roof of the anterior part of the pharynx. Each pad measures about 0 7 cm in length and about 0 5 cm in width and bears about 42 teeth

The dentition of *Mastacembelus* is polyphyodont, a new tooth originating from the base of a worn out tooth The teeth are highly specialised for catching the prey and also for preventing ts escape out of the buccal cavity

The Oesophagus

The bucco-pharynx, at its anterior end descends a l ttle and passes into narrow long tubular oesophagus which is bout 2·3 cm. long and about 0·4 cm in diameter In its natural position the posterior half of the oesophagus is covered over by the liver The pneumatic duct leading into the air bladder arises as a dorsal diverticulum of the oesophagus The mucosa of the oesophagus is raised into well pronou ced longitudinal folds continuous from the posterio part of the pharynx.

The Stomach

The stomach of *Mastacembelus* is a long nconspicuous structure, almost of the same diameter as the oesophagus It is bout 1 cm long ar d about 0·5 cm in diameter It bends posteriorly nto a J like structure the smaller arm of J representing the pylori tomach, while th main arm forms the cardiac stomacl T mucous lining of the cardia stomach is raised into almost straight longitudinal folds, while the pyloric region ha thick and more conspicuous mucosal folds.

The Intestine

The stomach leads into a short uncoiled almost straight tube, the intestine, lying in the posterior half of the body cavity Anteriorly it forms a U shaped loop with the posterior limb of the stomach.

The length of the intestine varies in the different fishes in relation to the body size of the fish. In *Mastacembelus* it has been estimated that the size of the intestine varies in direct proportion with the length of the fish. It has been found that the intestine of a full grown specimen is about 5·5 cm long and 0 4 cm in diameter The mucosal folds of the intestine are web-like and thus increase the absorptive surface of the intestine.

Anteriorly at the junction of the intestine with the stomach are given out a pair of intestinal or pyloric caeca (Fig 1) the caeca are small—about 6 mm long and 3 mm in diameter—blunt finger-shaped structures which are directed backward Its mucosa is thrown into folds known as caecal villi similar to those of the intestine.

The Rectum

Posteriorly the intestine passes insensibly into thin walled rectum which opens to the exterior through small slit like ventral anus situated in front of the anal fin There is a prominent ilio-rectal valve at the junction of the ilium and rectum. The mucosa of the rectum is almost smooth.

The Digestive Glands

The liver is a large unilobed solid mass dark brown in colour it lies above the posterior half of the oesophagus and the anterior half of the stomach to which it is firmly attached A large hepatic duct arises from the middle of the liver and opens into the gall bladder

The gall bladder is a conspicuous oval structure dark green in colour and is filled with a greenish fluid The common bile duct arises from the gall bladder and opens into the anterior part of the duodenum.

The pancreas of the fish is diffused in and around the liver

pH DETERMINATION

The determination of pH is an important aspect of the study of the process of digestion for different enzymes act optimally under different hydrogen-ion-concentrations. The pH of the different parts of the digestive tract and the associated glands has been tested both by capillary indicator and indicator paper methods

The average pH of the different parts of the normal feeding fish which were dissected soon after they were collected is tabulated on the next page

TABLE 1

pH of the different parts of the Alimentary Canal of Mastacembelus

Serial No.	Buccal cavity	Pharynx	Oesophagus	Stomach	Gall bladder	Pyloric caeca	Liver	Intestine	Rectum
1	6 5	6 8	6 7	6 4	6 9	6 4	7 5	6 7	6 7
2	6 6	6 8	6 8	6 5	6 8	6 7	7 4	6 4	6 9
3	6 5	6 7	6 9	6 7	6 8	6 6	7 3	6 6	7 0
4	6 4	6 9	6 8	6 5	6 9	6 5	7 4	6 5	6 8
5	6 6	6 7	6 7	6 6	6 7	6 5	7 5	6 5	6 8
6	6 5	6 8	6 8	6 6	6 8	6 6	7 1	6 5	6 9
Average pH	6 5	6 8	6 8	6 6	6 8	6 6	7 4	6 5	6 9

The above table shows that the medium in the buccal cavity stomach intestinal caeca and intestine is slightly acidic while the pH in the pharynx, oesophagus, gall bladder and rectum is nearer neutrality The medium in the liver is weakly alkaline.

A few fishes were starved for about 70 hours by keeping them in filtered water so as to clear off the alimentary canal from any food contents the pH of the parts of the gut was tested as before The results of these readings were found to be not very different from the normal feeding fishes as given in table I

Another set of fishes which were starved for about 80 hours were then fed on animal plankton so as to find out if the selective feeding has any effect on the pH of the alimentary canal the results were negative.

QUALITATIVE ESTIMATION OF ENZYMES

The extracts of the different parts of the alimentary canal were prepared as described earlier A few drops of the tissue suspension of the different parts of the gut of *Mastacembelus* were incubated with a few drops of different substrates at room temperature th control experiments were also set up in each case. These incubated solutions were tested after the interval of 24 hours, 48 hours and 4 days for any digestion of the substrates. For the determination of amylase, invertase, glycogenase raffinase inulinase and salicinase, Fehling's and Benedict s tests were performed while for maltase and lactase, osazone and Barfoed s tests were performed

The presence of lipase was tested by conducting experiments on condensed milk. Two drops of bromo-thymol blue were added to 25 ml of a 10% milk solution 1% sodium hydroxide solution was added until the solution turned light blue. One ml of blue milk solution was added to a few drops of the different extracts and the rest of the tubes were filled with toluene.

After a few hours of incubation the colour of the solution, wherever the lipase was present, changed to yellow. However the control experiments in each case gave negative results. The presence of lipase was further confirmed by experimenting with olive oil. Ten drops of olive oil were dissolved in 4 ml of absolute alcohol 4 ml of hot water was added to it. The mixture was then allowed to cool and 10 drops of phenol red were added. A few drops of 0·01 N NaoH were added to make the emulsion faintly pink. 2 ml of this mixture was incubated with 1 ml of each extract. Shortly the pink colour of the mixture changed to yellow thus confirming the presence of lipase.

The presence of proteases was investigated by incubating a few drops of the different extracts with 10 % gelatine solution. The gelatine was completely liquified in the cases where the enzyme is present in the control experiments the gelatine remained solid.

To summarise these results the following tables are given to demonstrate the results of these experiments with respect to the extracts from the different parts of the alimentary canal of *Mastacembelus*. The sign ++ means a vigorous reaction + a definite positive reaction ± traces of reaction while — indicates no reaction at all.

TABLE 2

Showing the presence or absence of Enzymes in the Liver

S No.	Substrate	Duration of reaction & extent of digestion			Control experiments	
		After 24 hrs.	48 hrs.	4 days	24 hrs.	4 days
1	1% starch soln	++	++	++	—	—
2	Saturated soln of glycogen	+	+	+	—	—
3	5% sucrose soln.	+	+	+	—	—
4	2% maltose soln.	—	—	—	—	—
5	2% lactose soln	—	—	—	—	—
6	1% raffinose sol	+	+	+	—	—
7	1% inulin soln.	+	+	+	—	—
8	1 salicin soln	±	+	+	—	—
9	10% gelatine soln	Dissolved in 8 hours			Remained solid	
10	Condensed milk etc	Colour changed to yellow			No change in colour	—

The above table shows that most of the carbohydrates are digested by the enzymes secreted by the liver cells and the cells of the pancreas which lies diffused with in the liver. However maltose and lactose are not digested. Both proteases and lipase are quite active.

Similar experiments were also set up with the extracts of intestinal caeca and the intestine, the results are tabulated in the following tables 3 and 4

TABLE 3
Showing the Results of the Experiments with the Caecal Extract

S. No.	Substrate	Duration of reaction & extent of digestion			Control experiments	
		24 hrs.	48 hrs.	4 days	24 hrs.	4 days
1	1% starch soln.	—	—	—	—	—
2.	Saturated soln. of glycogen	+	+	+	—	—
3.	5% sucrose soln.	—	—	—	—	—
4.	2% maltose soln.	—	—	—	—	—
5.	2% lactose soln.	—	—	—	—	—
6	1% raffinose soln.	—	—	—	—	—
7	1% inulin soln.	—	—	—	—	—
8.	1% salicin soln.	—	—	—	—	—
9	10% gelatine soln.	Got semi-digested			Remained solid	
10.	Condensed milk etc	No change in the colour			No change in colour	

TABLE 4
Results with the Intestinal Extract of Mastacembelus

S. No.	Substrate	Duration of reaction & extent of digestion			Control experiments	
		24 hrs.	48 hrs.	4 days	24 hrs.	4 days
1	1% starch soln.	—	—	—	—	—
2	Saturated soln. of glycogen	+	+	+	—	—
3.	5% sucrose soln.	—	—	—	—	—
4	2% maltose soln.	—	—	—	—	—
5	2% lactose soln.	—	—	—	—	—
6	1% raffinose soln.	—	—	—	—	—
7	1% inulin soln.	—	—	—	—	—
8.	1% salicin soln.	—	—	—	—	—
9	10% gelatine soln.	Became semi-liquid			Remained solid	
10	Condensed milk etc	No change in the colour			No change in colour	

The results tabulated in tables 3 and 4 show that only glycogenase and proteases are secreted by the caecal and intestinal cells of the fish.

Similar experiments were performed with the extracts of oesophagus, stomach rectum and gall bladder and it was observed that none of the digestive enzymes are secreted by the cells of any of these parts.

DISCUSSION

The fish *Mastacembelus pancalus* is a bottom feeder selecting its food from the substratum where it burrows. The intestine is comparatively very small and this can be interpreted in terms of diet. It has also been observed that there is some effect of the diet upon the length of the intestine which is mainly concerned with the absorption of digested food. Al-Hussaini (1947b) described the intestine of carnivorous forms, *Belone* and *Sphyraena* as small and almost straight and the carnivorous habit of *Mastacembelus* accounts for its small intestine and as suggested by Al Hussaini (1947b) is an adaptation to carnivorous habit The presence of pyloric-caeca as outgrowths of the intestine, compensates for this small size of the intestine. According to Rahimullah (1945) the caeca are characteristic of Actinopterygii and are supposed to perform the function of the storage of reserve food material and also secrete such digestive enzymes as diastase, lipase trypsine etc.

The small and inconspicuous nature of the stomach is another adaptation to its feeding habit as the fish is only a casual feeder—it has not to store the food in the stomach which is its primary function.

The investigations of pH in different parts of the alimentary canal of *Mastacembelus* show that the medium in the different parts of the gut is very similar to each other being only slightly alkaline in the liver while it is distinctly acidic in the intestinal caeca Bayliss (1935) and Al-Hussaini (1949b) also noticed that the medium in the stomach and other parts of the gut of teleosts is only slightly acidic. It has also been observed that the pH varies but little in the empty alimentary canal and that full of food contents.

The pancreas in *Mastacembelus* as also found by Babkin and Bowie (1928) in *Fundulus* is in a diffused form and extends into the liver Most of the enzymes exhibited by liver extract of the fish may be attributed mainly due to presence of pancreas around the liver in the diffused form.

Ishida (1936) found that many of the carbohydrate hydrolysing enzymes are present in most of the fishes with in the intestine but practically none could be traced in the intestine of *Mastacembelus*. It has also been observed by Agrawal and Singh (1963) that most of the carbohydrates are hydrolysed by the herbivorous fish *Colisa fasciata* These carbohydrases are not very active in the carnivorous fish *Mastacembelus* the proteases and lipase on the other hand, are more active in this carnivorous fish.

Summary

Mastacembelus pancalus is an elongated eel like fish which usually burrows under the soft substratum. It is predominantly carnivorous fish.

The alimentary canal of the fish consists of the buccal cavity, the oesophagus, stomach, intestine and rectum; paired pyloric caeca arise as small processes of the intestine at its junction with the stomach.

The study of pH shows that the medium in the buccal cavity, the stomach, pyloric caeca and intestine is weakly acidic while in the pharynx, oesophagus, gall bladder and rectum it is nearer neutrality. The pH in the liver is weakly alkaline.

Investigations on the qualitative estimation of digestive enzymes in the different parts of the gut revealed that many of the carbohydrases are secreted in the liver which also includes the diffused pancreas, while almost none are present in any part of the gut proper. Proteases and lipase are also quite active in the liver extract of the fish.

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UPTAKE OF NITROGEN PHOSPHORUS AND POTASSIUM BY CHLOROSIS AFFECTED CITRUS PLANTS DURING RAINY SEASON IN COORG

*N N. DIKSHIT[1] AND C. M. ABDUL MAJID[2]

Dieback of citrus trees widespread occurrence of chlorosis both general as well as interveinal and unproductiveness of the citrus trees has been partly attributed to the poor nutrient status of the soil in south India by Nara singharao (1948) Ramakrishnan (1954) Iyer and Iyengar (1956) and Maria kolandai and Dorairaj (1958)

General symptoms of chlorosis and die-back of mandarin plants of seedling origin as they occur in Coorg have been described by Dikshit (1958 a) who has also reported on the variations in the intensity of chlorosis as influenced by root stock and season He has also reported (1958 b) reduction in the intensity of chlorosis following sprays with some microelements and an increase in the intensity following irrigation treatments during the dry months Aiyappa and Dikshit (19) have reported differences in the macroelement constituents of the leaves from affected trees as compared to those of the healthy trees. They found that the affected leaves were always lower in the contents of N P K, Ca and Mg In the present observational study it was planned to find out if these plants could take up major elements N P and K from the soil under the conditions prevalent at Gonikoppal (South Coorg) This locality faces a somewhat heavy rainfall (1835 millimeters per year) and most of the precipitation occurs during the rainy season that is June to October It was observed earlier at this station that the intensity of chlorosis on the plants remains low during the months February to April. It starts rising from May and there is a well marked increase during the months of June, July and August. Thereafter a slight decrease in the intensity is noticed followed again by a rise. The highest figure for intensity is reached during the following December and January

In view of the observations reported above it was thought that the heavy rains might be playing some part in inducing the chlorosis either as a result of leaching of nutrients from the open textured soils due to rains or from the unhealthy condition of roots resulting from these heavy rains In the present study these two aspects were kept in view

Ten severely affected plants, growing in the orchard attached to the citrus die back research station Gonikoppal Coorg, (Mysore State) were selected for the study They were about 2 years old and were raised in

Present address 1 Assistant Officer's Specialist to Govt., U P Kanpur
2 Research Assistant, Citrus Dieback Research Station, Gonikoppal, Coorg.

nursery and planted in the field under comparable conditions. A basal dressing of 3 lbs. fertilizer mixture (1 lb. ammonium sulphate + 1 lb. superphosphate + 1 lb. muriate of potash) along with 128 lbs of Cattle manure were uniformly applied per tree. Thereafter different fertilizers as given in table 1 were added at monthly intervals. The fertilizers were added around the tree in a circular area, about 8 feet in diameter with tree in centre. The doses were kept rather high so that enough nutrients may be available to the plants even after leaching.

TABLE 1

Showing details of the manurial treatments given to different sets of the plants

(Dose per plant)

Treatment No.	Fertilizer	Monthly quantity applied	Months when applied	Total fertilizer	Total nutrient
1	Ammonium sulphate (20% N)	2 lbs.	June July August & September	8 lbs. N	1·6 lbs.
	Urea (44% N)	1 lb.		4 lbs. N	1 8 lbs.
3	Superphosphate (16% P_2O_5)	5 lbs.		20 lbs. P_2O_5	3·2 lbs.
4	Muriate of Potash (60 K_2O)	1 lb.		4 lbs. K_2O	2 4 lbs.
5	Control	3 lbs. of mixture containing N P and K, lb of cattle manure			128 lbs.

Samples of the leaves of comparable age and soils were collected from the treated plants during the months July September and October. They were analysed for major elements. The figures given in the tables indicate means of the three values thus obtained and are taken to represent the prevalent status of the major elements in the soils and the leaves during the period of observations. The figures are given in the tables 2 and 3.

RESULTS AND DISCUSSIONS

Nit s During the course of this study 1·6 lbs. of nitrogen were given to t o plants in four months split in four equal parts. Although the usual recommended dose of N for bearing citrus trees *is 1 to 2 lbs* of N for one year the above heavy dose was given to assure adequate quantity of N in soil. The mea nitrogen content of the soils was found to be 0·216 which is 2 higher than that observed under control plants. It is thus seen that a higher level of n trogen was successfully maintained by adding sulphate of ammonia repeatedly. The soil under control plants contained *only* 0·1 N

In another set of two plants the nitrogen was applied in form of urea as soil application. The plants received 1 8 lbs of nitrogen during the months. It is seen f om the figures given in table 2 that the soil plants, which received nit ogen in form of urea had only 0 161%

which was lower than that found under the control plants The higher level of nitrogen following application as ammonium sulphate may be because in this form it is known to be retained in the soil in the exchange complex for a limited period, a fact also reported by Tidmore and Williamson (1932) The adsorbed ammonia is slowly released and utilised gradually by the plant roots Comparatively less level of N under the plants which received urea may be because it is known to be better utilized if it is converted into ammonium carbonate in soil. Nitrogen is lost by leaching as organic fertilizer does not participate in the nutritional process through the exchange complex but is utilised directly by the roots

TABLE 2

Showing macroelement content of soils following application of different fertilizers

(Per cent oven dry basis)

	N as Amm. sulphate	N as Urea	P as super phosphate	K as muriate of potash	Control N,P,K mixture 5 lbs. Cattle manure 128 lbs.
N	0 216	0 164	0 177	0 198	0 170
P_2O_5	0 077	0 055	0 091	0 065	0 016
K_2O	0 077	0 155	0 042	0 144	0 053
CaO	0 289	0 254	0 150	0 178	0 257
MgO	0 268	0 182	0 273	0 251	0 231

N B —Fertilizers applied per plant as shown in table 1

TABLE 3

Showing macroelement content of leaves collected from plants receiving different fertilizer treatments

(Per cent oven dry basis)

	N as Amm sulphate	N as Urea	P as super phosphate	K as muriate of potash	Control N,P,K mixture @ 5 lbs cattle manure 128 lbs.
Ash	5 867	5 829	7 298	6 399	7 885
N	2 720 O	2 437 O	2 399 L	2 294 L	2 252 L
P	0 141 O	0 085 L	0 111 L	0 156 O	0 1003 L
K	0·693 L	0 718 L	0 867 L	0 775 L	0 967 L
Mg	0 432 O	0 459	0 542 O	0 378 O	0 495 O

N B —Rate of fertilizer application per plant was as in Table 1

O Optimum range as per classification by Reuther and Smith.

L Low range the element needs to be supplied to the trees to keep the optimum production

The nitrogen content of leaves is given in table 3. The figures show the highest content of nitrogen in the leaves from plants which received N as ammonium sulphate and the value was 2% higher than that of control. It is interesting to note that although the nitrogen content of soil near the urea treated plants was lower than the controls the N content of the leaves of urea treated plants was higher than the controls. This naturally indicates that the plants utilised N even in form of urea though not to the same extent as from ammonium sulphate. Loss of N from soil when it was applied in form of urea is indicated from the figures of soil analysis given in table 2. It does appear from the figures of analysis of leaves give in table 3 that some N was utilized by the plants

The figures given in table 3 were further examined in the light of the standards of classification laid down by Reuther and Smith (1954). The standards are for citrus sinensis but the planting material in Coorg is citrus reticulata. Thus the standards laid down by Reuther and Smith are applicable to the leaves collected in Coorg only to a limited extent. However they are being discussed for comparison without basing the conclusions on them.

According to the classification of the nutrient status of the plants based on the analysis of leaves it is seen that the N content of the leaves of the plants which received nitrogen in form of ammonium sulphate as well as urea was in the range of optimum while that of leaves from control plant was lower. It is thus indicated that the plants are capable of using nitrogen from soils even during the rainy season provided it is present in the soil. It is also indicated that by timing the application of fertilizers properly it is possible to increase the content of N in the leaves

The repeated doses of ammonium sulphate not only increased nitrogen content of the leaves but also that of P and Mg. Thus P and Mg content of the leaves was also in optimum range when nitrogen was applied as ammonium sulphate. Calcium and potassium content of the leaves, however continued to be low. Repeated doses of nitrogen in form of urea however induced optimum content in leaves regarding nitrogen and magnesium only

(ii) *Phosphorus* In the present observational trial it was applied at the rate of 20 lbs of superphosphate per plant split into four applications. Following the application of superphosphate to the soil the P_2O_5 content of the leaves was seen to increase But it could not reach the optimum range In this connection it [illegible] time to note that when ammonium sulphate was applied the content of N and P in the leaves rose to the optimum level although no special attempt [illegible] made to supply phosphates. On the other hand when high dose of superphosphate was applied without additional nitrogen neither N nor P in the leaves was found to be in the optimum range.

It can therefore be concluded that Nitrogen is the most important factor which affects the uptake of N as well as phosphatic nutrients. Applicat[illegible]

of phosphate alone failed to induce uptake of either of the two and can hardly be considered useful from that point of view

(iii) *Potassium* It was applied to the plants in the form of muriate of potash. Following the addition of muriate of potash the K_2O content of the soil was noticed to rise. Similarly an increase in the K content of the leaves was also observed. But the K content of the leaves never reached the optimum level.

The low status of P and K in the leaves in spite of addition of the fertilizers containing P and K may to some extent be due to the low level of N in the leaves. It was seen that when adequate N level is maintained in the leaves the uptake of other major elements is also improved within certain limits. It thus appears that the optimum level of N in the leaves is probably partly responsible for proper uptake of other macro-elements by plants under field conditions in Coorg

The present observational studies indicate that the current method of fertilization of citrus trees in Coorg is probably not able to build up adequate level of N P and K in leaves. Adequate quantities of P and K when applied without N were also not utilised by the plants to the optimum extent. Repeated doses of N in form of ammonium sulphate during rainy season, however gave optimum of N P and Mg in the leaves. In view of these observations beneficial results can be expected if the basal application of fertilizers as given under treatment 5 is supplemented by repeated applications of nitrogen as ammonium sulphate. Further work is in progress.

ACKNOWLEDGEMENT

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PHYSIOLOGICAL STUDIES ON SALT TOLERANCE IN CROP PLANTS

XX. Influence of sodium chloride on the contents of carbohydrates and nitrogen in seedlings of wheat and gram (*Cicer arietinum* L.)

*S N Bhardwaj[1] and I M Rao[2]

Botany Department Agra College Agra

Introduction

Accumulation of the neutral salts (chloride sulphate, etc.) in the soil leads to increase in the osmotic pressure of the soil solution which retards plant growth thus there has been some tendency to regard the salinity injury to be due to the osmotic effect of the soil solution (Magistad *et al* 1943 Hayward and Spurr 1943 Magistad 1945 Hayward and Wadleigh 1949 Bernstein and Pearson 1954 Brown and Hayward 1956 and Hayward and Bernstein 1958) It is, however noted that individual components of the soil solutes may have as a result of their absorption and accumulation, some specific toxic effects on plants. Some plants are more sensitive to chloride than sulphate (Eaton 1942 Russel 1950 Bhardwaj and Rao 1955 and Hayward and Bernstein 1958)

Physiological investigations relating to the effect of above salts on plant metabolism have been attempted by comparatively a few workers (Garner *et al* 1930 Baslavskaja 1936 Eaton 1942, Sarin and Rao 1958, Bhardwaj 1959 Bhardwaj and Rao 1960 etc.) and the depressing effect has generally been attributed to ion accumulation in the cells to toxic concentration leading to disturbance in normal functioning of the plant. Attempt has been made in the present study to investigate the influence of a harmful concentration of NaCl (0·6%) on early seedling metabolism in wheat and gram. It was intended to find out the differences between two crops and their varieties and to correlate the same with their relative tolerance to the salt.

Methods and Materials

Two varieties of wheat (N P 165 and C. 591) and two of gram (N P 28 and T 87) were employed in the present experiment wheat N P 165 and gram N.P 28 were earlier in flowering by about ten days than the remaining two types. Seeds were germinated and seedlings were grown in large petri dishes on moist filter paper kept at 22°C in darkness The treated set received 0 6% NaCl solution and the controls distilled water

At 96 hours after sowing seedlings were analyzed colorimeterically for reducing and non-reducing sugars and total and protein nitrogen adopting the methods suggested by Snell and Snell (1955) The ash content was determined following the A.O A C. method

1 Present address Botany Division Indian Agricultural Research Institute, New Delhi-12.

2 Professor and Head of Botany Department, Sri Venkateswara University Tirupati (Andhra State).

For the data recorded on dry weight of the seedlings and loss in dry weight (0-96 hours) standard errors were calculated and are included along with the 'means' in the text (Table 1). Analysis of variance method was adopted for statistical interpretation of the data on chemical analysis of the seedlings and the critical differences at 5 per cent probability were calculated, wherever the treatments were significant.

Results and Discussion

In order to ascertain the depressing influence of sodium chloride supplied to the seedlings upto 96 hours seedling dry weight and loss in dry weight were calculated and are furnished in table 1.

Table 1

Effect of 0.6% NaCl on seedling dry-weight and loss in dry-weight in wheat and gram

(Mean of 10 values)

Observation	Wheat		Gram	
	N P 165	C. 591	N P 28	T. 87
Seedling dry-weight in mg at :—				
0—hours	41±3	38±3	149±8	183±10
96—hours				
(i) Control	35±4	29±4	121±10	161±13
(ii) 0.6% NaCl	38±3	34±5	135±10	163±11
Loss in seedling dry-weight (0–96 hours) in mg :—				
(i) Control	6±1.0	9±1.1	28±6.2	22±4.8
(ii) 0.6% NaCl	5±0.8	4±0.8	14±5.3	18±3.4
(iii) % on control	50	44	50	82

It is seen that seedling dry weight at 96 hours compared to the control was higher in the series supplied with 0.6% NaCl although the variations in respect of crops and their varieties were quite conspicuous. The two varieties in wheat suffered almost equally while in gram T 87 was found to be relatively more tolerant.

Observations on the contents of sugars (reducing and non-reducing), nitrogen and ash of the seedlings have been expressed as percent gm on oven-dry weight as well as per seedling basis and are presented in table 2.

TABLE 2

Effect of 0 6% NaCl on the contents of sugar nitrogen and ash of wheat and gram seedlings

Observation	Wheat				Gram			
	N P 165		C. 591		N P 28		T 87	
	NaCl supplied to the seedlings 0-96 hours (% sol tion)							
	0 0	0 6	0 0	0 6	0 0	0 6	0 0	0 6
(i) On dry weight basis :—								
Reducing sugars	8 40	4 0	15 20	8 90	6 59	0 16	1 75	0 55
Non-reducing sugars	3·40	4·49	3 55	3 55	4 78	6 59	4 52	4 72
Total Nitrogen	2 18	2 43	1 60	1 91	2 77	3 49	2 69	2 69
Protein Nitrogen	1 50	1 43	1 15	0 81	2 30	1 98	2 30	2 37
Ash	2 00	1 90	30	2 75	3 53	3 75	3 60	3 90
(ii) On per seedling basis (in mg)								
Reducing sugars	2 94	1 60	4 41	3 03	0 71	0 22	2 82	0 91
Non-reducing sugars	2·04	3 23	1 03	1 21	5 78	8 90	7 28	7 79
Total Nitrogen	0 76	0 93	0 46	0 66	3 35	4 71	4 33	4 44
Protein nitrogen	0 53	0 54	0 53	0 51	2 78	2 67	3 70	3 91
Ash	0 70	0 72	0 67	0 91	4 30	3 06	6 12	6 41

Critical difference at 5% probability

Observation/Due to	Wheat			Gram		
	Salt	Variety	Interaction	Salt	Variety	Interaction
(i) On dry weight basis						
Reducing sugars	2 32	2 32	N S.	0 42	0 42	N.S.
Non-reducing sugars	N.S	1 81	2 32	0 73	0 73	1 01
Total Nitrogen	0 24	0 24	N.S	N.S	0 24	0 24
Protein Nitrogen	0 08	N.S.	0 11	0 17	N.S.	0 24
Ash	N.S	N S	N S	N.S	N.S	N S
(ii) On per seedling basis						
Reducing sugars	1 39	1 39	N S	0 52	0 2	N S
Non-reducing sugars	0 42	0 42	0 60	N.S	0 47	N S
Total Nitrogen	N S.	N S.	N.S	N.S.	N.S	N S
Protein Nitrogen	0 67	N.S	0 11	0·21	N.S.	N S
Ash	N.S	0 20	N.S.	N S.	0 98	N S

N S Statistically non-significant

From the above it seen that in the two crops, as a result of the salt treatment there was an apparent increase in non reducing sugars and a decrease in protein-nitrogen and reducing sugars. Varietal differences were also apparent especially in respect of ash content and non-reducing sugars.

Effect of chloride on plant metabolism has been investigated by a few workers Garner *et al* (1930) reported a pronounced dissipation of malic acid as a result of chloride accumulation in tobacco leaves amylolytic activity was disturbed and the leaves became gorged with starch. Buslavskaja (1936) observed that accumulation of chloride ion (in potato leaves) interferes with photosynthetic mechanism *i.e.* causes a reduction in chlorophyll contents and consequently a reduction in total carbohydrate contents there was a definite increase in starch/sugar ratio. Gauch and Eaton (1912) also noted in barley shoots that the concentration of sucrose fraction was markedly affected by salt treatment chloride content was subsequently higher in chloride plants than the control' plants.

Earlier studies by the authors (Bhardwaj and Rao 1960 and Bhardwaj 1961) also reveal that supply of chloride (as NaCl) to wheat and gram seedlings upto four days in toxic concentration of 0·6 per cent resulted in lowering of respiration rate and hydration percentage and consequently in depression of the early growth of roots and of shoot. In the present study the influence of the salt (chloride) on fractions of carbohydrates and nitrogen and ash contents of the seedlings in wheat and gram is quite apparent. Thus it can be inferred that chloride results in an initial decrease of water absorption by the roots and also on accumulation (as indirectly indicated by higher ash content of the salt fed seedlings) it interferes with respiration, protein-synthesis and carbohydrate metabolism. Support to the above assumption is sought from the data furnished in table 3 below as well as from the results reported elsewhere.

TABLE 3

Effect of NaCl (0 6% solution) on early seedling metabolism in wheat and gram.
(Expressed as percentage on control *i.e.* distilled water supply)

Crop and variety	Root growth	Respiration rate (Q2)	Hydration percentage	Loss in dry weight	Ash content	Reducing sugars	Protein nitrogen
Wheat N P 165	53	81	86	50	95	50	95
Wheat C. 591	58	71	93	44	170	59	79
Average	56	76	90	47	103	55	87
Gram N P 28	53	81	97	50	112	27	86
Gram T 87	50	67	89	82	103	32	103
Average	53	6	93	66	108	29	95

Data reported by Bhardwaj (1961)
Data reported by Bhardwaj and Rao (1960)

The conspicuous reduction in reducing sugars in the treated seedlings as also, to a lesser extent in protein nitrogen, are perhaps related to the adverse effect of the salt on growth. Decrease in reducing sugar is more evident in gram compared to wheat thus reducing sugars at the seedlings stage can give a fair indication regarding the depressing effect of the salt.

SUMMARY

Influence of sodium chloride at 0·6 concentration on fractions of carbohydrates and nitrogen and ash content of four days old seedlings of two varieties of wheat (N P 165 and C. 591) and two of gram (N P 28 and T 87) was estimated. The results indicate that supplying NaCl leads to an increase in non-reducing sugars and a decrease in reducing sugars differences in respect of the above were apparent in the two crops as well as in between the varieties.

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THE PREPARATION OF ORGANO-MERCURY COMPOUNDS AS MILDEW AND ROT PROOFING AGENTS

Vishwa Nath Nigam
The Chemical Laboratories D. A. V. College, Kanpur

One of the most serious problem that has been faced by the Military persons and by the Textile Industry from time immemorial has been that of Rot and Mildew. The textile materials are attacked by Micro-organisms all through the various stages of manufacture from raw material to finished goods and there are further loss suffered by distributors as well as by users and when the goods are in use. Perhaps the heaviest losses sustained by the Industry have occured in materials for export in grey state as several months may elapse between the time the material leaves the Mill and the time it arrive at its destination.

World wide political conditions and threat of hostility made the rot and mildew attack on cellulosic materials a matter of serious strategic importance for our defence especially in North Eastern Frontier area and Assam. It is obviously to be expected that in these area equipment with a predictable service life of months or years, when used in temperate climate becomes inoperable in a matter of few weeks.

India is a tropical country and textile materials form an important item of Industry required both for civil and military uses. India's Textile Industry is also the back bone of industrial activities earning much foreign exchange which is very vital for India's economy. Therefore improvements and development of newer preservatives which will prevent the Rot and Mildew deterioration of textile materials under the conditions prevailing in this country and enhance the life of cotton and cellulosic materials both in use and storage is of higher significance for India's economy and Military needs.

Dyes belonging to different groups are used as fungicide and bactericide. Quite prominent among these are some azodyes as Scarlet Red Dimazon Pyridium, Chloroazodin Prontosil, Rubiazol and Azo-sulfamide.

Mercury also possesses intrinsic fungicidal and bactericidal properties. The introduction of mercury in various aromatic nucleus has been found to greatly augment these properties.

Although the organo-mercury compounds are strong fungicide and bactericide but their use in textile industry is not possible due to the insolubility of these compounds in water and various organic solvents. On the

Summary of the thesis submitted to the Agra University for the Degree of Doctor of Philosophy

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uniformly upto the desired concentration and to impart positive and lasting bacterio-static and fungistatic properties to the material suited to all the climatic conditions of the world. Instead of these requirements they must also fulfil the following desired properties. The treatment should not impart toxic or irritating properties to the fabric and should be compatible with alkali soluble cellulose finishes, starch finishes sizing water proofing, moth proofing and fire proofing. It should impart no objectionable odour should not effect the physical properties of the fabric, should not be volatile or water soluble and should not increase the inflamability of the goods.

Experimental

We have developed the process for the preparation of the following mercurated compounds

1. p-Chloromercuri phenol.
2. 2-Chloromercuri p-cresol.
3. Mono-mercurated-α-naphthol.

The above mentioned compounds and the o-chloro mecuriphenol were coupled with different diazotized bases. The mercurated dyes so prepared are given on page No. 461-462. Besides these their corresponding unmercurated compounds for test were also prepared. The list of the same is given on page No. 463-464. These compounds were applied on the Poplin No. 090007 of Delhi Cloth Mills as per method given on page No. 227-228.

The dyed fabric were then tested for dye fastness (Light washing and rubbing) and resistance to Mildew and Rot.

I. *Dye fastness*

1. The fastness to light was tested in Atlas Fadeometer as per details given on page No. 254.
2. The fastness to washing was tested as per standard wash wheel test No. 2 and wash wheel test No. 4 as per method given on page No. 255.
3. The fastness to rubbing was tested as per method given on page No. 257.

II. Resistance to mildew and rot proofness was determined by Pure Culture Test and Soil Burial Test.

1. Pure Culture Test was performed as per method given on page No. 296-300.
2. Soil Burial test was performed as per method given on page No. 385.

The interesting results of *o-Chloromercuriphenol* gave encouragement towards the preparation of its colourless derivatives for universal application

as fungicide and bactericide. To achieve this object various imides (Succinimide -α phenyl succinimide, phthalimide 3-Nitro phthalimide and 4-nitrophthalimide) were prepared. The mercurated derivatives of the above mentioned imides were prepared by reacting them with mercuric acetate as well as by condensing them with o-chloromercuri phenol. The list of the compound so obtained is given on Page No 464

These compounds are under test for resistance to Mildew and Rot.

CONCLUSION

For the search of suitable materials which would function as mildew and rot proofing agents for protecting textile material from their attack a number of suitable azo dyes have been prepared and described in this thesis. From a study of their dyeing properties and an examination of their rot proofing and fungicidal properties the following observations have been noted.

(a) The introduction of the mercury in azo dye had given a new field of work. It is evident from the table No VI that dye fastness (Light, washing and rubbing) had much improved than their corresponding unmercurated ones except in (i) 2 Methoxy 5-nitro-2 hydroxy-3 -chloromercuri 5 methyl azobenzene (ii) 2 Nitro-2 hydroxy 3 -chloromercuri-5 -methyl azobenzene (iii) Sodium 2 hydroxymercuri 4 (2 -nitro-4 methoxy-benzeneazo) -α naphthol (iv) Sodium 2 hydroxy mercuri-4 (2 -nitro-benzeneazo)-α Naphthol and (v) 2-chloromercuri 4-(4 nitro-benzeneazo)-α naphthol.

(b) Mercury has not only increased the dye fastness but has also improved their shade. The dyes are bright deep as well as attractive than their corresponding unmercurated compounds. The comparative account of the shade and dye fastness is shown in folder containing the controls and tested pieces attached in the thesis from page No 266 to 295

(c) The importance of these compounds is further enhanced as they can be function as mildew and rot proofing agents for protection of textile material. The degree of their effectiveness is well illustrated in Graph Nos. II & III and table Nos. 88-95 It is clear from the above data that the introduction of mercury in azo dye helps to impart mildew and rot proofness to the textile treating fabric.

No doubt the azo dyes which are here described are not very fast to washing but they could be used where the degree of fastness to washing required upto the order of wash wheel test No. 2 i.e. Canvas, Cordages tape newar lining packing materials etc.

The order of their effectiveness of all these mercurated dyes as mildew and rot proofing agents is given on page

MIXED CULTURE

Series No. I

2-Methyl-3-chloromercuri-4-hydroxy azobenzene > 2 Methoxy 5-nitro-3-chloromercuri-4-hydroxy azobenzene > 4 Nitro-3-chloromercuri-4-hydroxy azobenzene > 4 Methyl-3-chloromercuri-4 hydroxy-azobenzene > 2-Nitro-4 methoxy 3-chloromercuri 4 hydroxy-azobenzene > 2-Methoxy 4-Nitro-3-chloromercuri-4-hydroxy-azobenzene and 2-chloromercuri-4-(2'-methoxy-4-nitro benzeneazo)-6-(2''-methoxy-4-nitro-benzeneazo) phenol > 2-Nitro-3-chloromercuri-4-hydroxy azobenzene > 3-Nitro-3-chloromercuri-4 hydroxy-azobenzene > and the following compounds are not at all effective

(i) 2 Methoxy 5-chloro-3-chloromercuri-4-hydroxy azobenzene and (ii) 2 Methyl-4-chloro-3-chloromercuri-4 hydroxy azobenzene.

Series No. II

2 Methoxy-5-nitro-2'-hydroxy 5-chloromercuri-azobenzene > 4-Nitro-2-hydroxy 5'-chloromercuri azobenzene > 2 Nitro-4-methoxy 2 hydroxy 5-chloromercuri azobenzene > 2-Methyl 2-hydroxy-5-chloromercuri azobenzene > and the following compounds are not at all effective.

(i) 2 Methoxy-4-Nitro 2-hydroxy 5-chloromercuri azobenzene (ii) 2 Nitro-2'-hydroxy 5-chloromercuri azobenzene (iii) 3 Nitro 2'-hydroxy 3'-chloromercuri-azobenzene (iv) 4-Methyl 2 hydroxy-5-chloromercuri-azobenzene (v) 2-Methoxy-5-chloro-2 hydroxy-5-chloromercuri-azobenzene (vi) 2 Methyl-4-chloro-2' hydroxy 5-chloromercuri-azobenzene.

Series No. III

2 Methoxy-4-nitro-2 hydroxy 3-chloromercuri-5-methyl-azobenzene > 4-Nitro-2' hydroxy-3-chloromercuri-5-methyl azobenzene > 2 Methyl-4-chloro-2'-hydroxy-3-chloromercuri 5-methyl-azobenzene > 2 Methyl-2'-hydroxy-3 chloromercuri 5 methyl-azobenzene > and the following compounds are near about similar in their effectiveness

(i) 2 Methoxy 5-nitro-2' hydroxy-3 chloromercuri 5 methyl-azobenzene (ii) 2 Nitro-4-methoxy 2-hydroxy-3 chloromercuri 5-methyl-azobenzene (iii) 2 Nitro-2' hydroxy 3-chloromercuri-5-methyl-azobenzene (iv) 3-Nitro-2 hydroxy-3-chloromercuri-5-methyl-azobenzene (v) 5-Methyl-2-hydroxy-3 chloromercury-5-methyl-azobenzene (vi) 2 Methoxy 2-chloro-2-hydroxy 3 chloromercuri-5 methyl azobenzene.

Series No. IV

2-Chloromercuri-4-(4-nitro-benzeneazo)-α-naphthol > Sodium 2-hydroxy mercuri-4(3-nitro-benzeneazo)-α-naphthol > Sodium 3-hydroxymercuri-4-(2'-methyl benzeneazo)-α-naphthol > Sodium 2-hydroxymercuri-4-(2'-methoxy-4-nitro-benzeneazo) > α-naphthol > Sodium-2 hydroxymercuri-4-(2-nitro-ben-

zeneazo) α naphthol > Sodium 2 hydroxymercuri-4 (2'-methoxy 5 -chloro-benzeneazo) α -naphthol > Sodium 2-hydroxymercuri-4-(2 methyl-4 -chloro-benzeneazo) α naphthol > Sodium 2 hydroxymercuri-4-(2'-methoxy 5 -nitro-benzeneazo) α naphthol > Sodium 2-hydroxymercuri-4-(2 -methoxy-4 -nitro-benzeneazo) α -naphthol.

SOIL BURIAL

Series No I

4-Methyl 3 -chloromercuri-4 -hydroxy-azobenzene> 2 Methoxy-4-Nitro-5' chloromercuri-4 -hydroxy azobenzene and 2-chloromercuri-4-(2 -methoxy-4 -nitrobenzeneazo)-6-(2 methoxy-4 nitro-benzeneazo) phenol > 2 Methyl 3 -chloromercuri-4 hydroxy azobenzene > 2 Methoxy 5- nitro-3 chloromercuri-4 hydroxy azobenzene > 2-Nitro-4 methoxy 3 -chloromercuri-4 -hydroxy-azobenzene > 2 Methoxy 5-chloro-3 -chloromercuri-4 hydroxy-azobenzene> 2-Nitro-3 -chloromercuri-4 hydroxy-azobenzene > 3 Nitro-3 -chloromercuri-4 -hydroxy-azob nzene > and the following compounds are not at all effective. () 4 Nitro-3 -chloromercuri-4 hydroxy-azobenzene and 2 Methyl-4-chloro-3 -chloromercuri-4 -hydroxy azobenzene.

Series N II

2 Methoxy-4 Nitro-2 -hydroxy 5 -chloromercuri-azobenzene > 4-Nitro-2' hydroxy 3 -chloromercuri-azobenzene > 2 Nitro-4-methoxy ? hydroxy 5 -chloromercuri azobenzene > 4-Methyl 2 hydroxy 5 -chloromercuri-azobenzene > 2 Methyl 2 hydroxy 5 -chloromercuri-azobenzene > 2 Nitro-2 -hydroxy 5 chloromercuri azobenzene > 3-Nitro-2 -hydroxy-5-chloromercuri-azobenzene > 2 Methoxy 5-Nitro-2 -hydroxy 5 -chloromercuri-azobenzene and the following compounds are not at all effective. (i) 2 Methoxy 5-chloro-2 -hydroxy 5 -chloromercuri-azobenzene and 2 Methyl-4-chloro-2'-hydroxy-5 -chloromercuri-azobenzene

Series N III

4 N tr hydro y 3 -chloromercuri 5 methyl azobenzene>2 Methoxy-4-Nitro-? hyd xy 3 -chloromercuri 5 -methyl-azobenzene>4 Methyl -hydroxy 3 -chloromer ury 5 methyl-azobenzene > 2 Nitro-4 Methoxy 2'-hydroxy 3 chl rom uri -methyl azobenzene > 2 Methyl-4-chloro-2 -hydroxy 3'-chloromer uri m th l azobenzene >3-Nitro-2 hydroxy 3 -chloromercuri 5 methyl-azoben n 2 M thoxy 5-nitro-2 hydroxy 3 -chloromercuri 5 -methyl azobenzen 2 N tro- hydroxy 3 -chloromercuri 5 -methyl azobanzene The following mp nd ar not at all effective. (i) 2 Methyl ? -hydroxy 3 -chloromercuri 5 methyl zobenzene and (ii) ? Methoxy 5-chloro-? -hydroxy-3 chloromercuri -methyl azobenzene.

Series No II

Sodium Hydroxyme curi-4 (2' nitro-benzeneazo)-naphthol > 2-hydroxymercuri 4 -methoxy 5 -nitro-benzeneazo)- α -naphth l >

2-hydroxymercuri-4-(3-nitro-benzeneazo) -α- naphthol > Sodium 2-hydroxy mercuri-4-(2-methoxy-4-nitro-benzeneazo -α naphthol > Sodium 2-hydroxy mercuri-4-(2 Nitro-4-methoxy-benzeneazo) naphthol > 2-chloromercuri-4-(4 nitro benzeneazo) -α naphthol and the following compounds are not at all effective Sodium-2-hydroxymercuri -4 (2-methyl benzeneazo) α-naphthol (ii) Sodium-2 hydroxymercuri-4-(4 methyl-benzeneazo) α naphthol (iii) Sodium 2-hydroxymercuri-4 (2 methoxy 5-chloro benzeneazo)-α-naphthol (iv) Sodium 2-hydroxymercuri-4-(2 methyl-4-chloro-benzeneazo)-α-naphthol.

The most important thing is that we have *discovered and developed a new process for the application of mercury in the form of organomercury compounds (a s dyes) to textile materials. The new process is easy and economical to apply uniformly upto the desired concentration. The process requires no binding agent thus the physical properties of the material remains unaltered.* These developed mercury compounds are also nonvolatile, odourless and do not increase the inflamability of the goods.

It appears that these mercurated compounds should not impart toxic or irritating properties to the fabric as the mercury is in the organomercury form (no actual test has been carried out to evaluate this properties). These compounds should be compatible with alkali soluble cellulose finishes, starch finishing and sizing water proofing. (No actual test has been performed to assess the above mentioned properties).

We have also developed the method for the preparation of p-chloromercuri phenol, 2-chloromercuri p-cresol and monomercurated-α-naphthol.

Mercurated derivatives of imides were also prepared and their fungicidal and bactericidal properties are under test.

THE BEHAVIOUR OF PARAMAGNETIC IONS IN SINGLE CRYSTALS OF SOME SALTS OF RARE EARTH AND IRON GROUP OF ELEMENTS*

SATISH CHANDRA MATHUR†

Agra College Agra

INTRODUCTION

The behaviour of paramagnetic ions in crystals is strikingly different from free ion behaviour In crystals the paramagnetic ion is surrounded by diamagnetic ions regularly arranged about it These diamagnetic neighbours produce an electric potential of a symmetry depending upon the distribution of charges This crystalline electric potential removes partially the degeneracy of the ground state. Study of this type of Stark pattern should therefore give us information about the nature of the crystalline forces These investigations can be done by either of the following methods

(1) Optical absorption measurements

(2) Paramagnetic resonance absorption measurements.

(3) Magnetic susceptibility measurements

Paramagnetic resonance techniques can give us information only about energy levels for which the energy separation is of the order of 0·1 cm^{-1} while optical absorption can provide information for energy seperation both, low and high. The splitting of the lowest energy levels is often related to the nature and spacing of the excited states and hence all the techniques (optical absorption, paramagnetic resonance absorption and magnetic susceptibility measurements) may yield interrelated results Consequently studies on mag netic susceptibilities are eminently suitable for getting information about the energy levels of the ions in crystals and that alternatively the ions may be regarded as probes measuring the crystalline electric field and have therefore engaged the attention of a large number of investigators

As is well known in the iron group of metal ions, M^{++} and M^{+++} all the inner levels are already full. The 3d shell of the ions which are getting progressively filled up are the outer most ones, since those occupying the 4s shell in the free atom have been removed in the formation of the ion in the crystal. Hence in the case of iron group the d-electrons are exposed directly to the effect of electric field which is in general strong and asymmetrical. Effect of these asymmetrical fields is to remove the orbital degeneracy But due to spin orbit coupling the quenching of orbitals is only partial. The

* Summary of the thesis submitted to the Agra University for the degree of Doctor of Philosophy in 1961

† Present address Delhi Polytechnic Delhi

problem of Stark splitting of the energy levels of ions under the influence of crystalline electric fields of different symmetry has been worked out by Bethe[1] Van Vleck[2] Penney and Schlapp.[3] The number of Stark levels and their relative seperations will depend on the strength and symmetry of the crystal field.

From the point of view of the influence of the electric fields on the energy levels of the ions, the environmental condition in the neighbourhood of these ions can be broadly divided into three classes.

(*a*) Conditions typified by those occuring in the salts of iron group of elements. In this case 3d-electrons are the outer most and hence the electric field effect will be much pronounced. As a matter of fact the field is strong enough to break L-S coupling, so that L and S must be seperately quantised.

(*b*) Conditions typified by the rare-earth ions in which the influence of the fields on the energy levels is greatly reduced by the shielding action of the outer shells of electrons, so in the study of the electronic structure of these ions we may treat each (L-S) multiplet seperately and consider the splitting of the multiplets due to the crystal field. In this case J remains a good quantum number and spin orbit coupling gains more importance than in previous case.

(*c*) Conditions as typical of the covalent complexes. Here the electric fields involved are very strong and their effects on the energy levels are very drastic.

The theoretical technique to be employed in the investigation of energy levels of these ions will therefore be different for different environmental conditions. A brief outline of the theoretical techniques has been discussed in the theoretical part of the thesis (Mathur S. C. Ph. D. thesis 1961 Agra University) under the heading "Influence of Crystalline Electric field on paramagnetic susceptibilities.

Studies on magne-crystallic action in ions of iron group and rare-earth group of metals have been carried out by many investigators. Guha[4] reported the temperature variation of anisotropies and principal susceptibilities of a number of iron group salts. Of these salts cupric acetate monohydrate received much attention because it exhibited an anomalous behaviour. Crystals of cupric acetate monohydrate were thoroughly investigated by paramagnetic resonance method by Bleaney and Bowers[6] and Abe and Shimada while Fox et. al.[7] and Figgis and Martin[4] studied the variation of mean magnetic susceptibility of cupric acetate monohydrate with temperature from measurements on crystal powders. Niekerk and Schoening,[3] did an investigation of the crystal by X-ray analysis. Though all the magnetic measurements agree at high tempera- X-ray data for cupric tures, show much discrepancy at lower temperatures.

formate tetrahydrate pointed to the probability of some abnormal behaviour of the crystal. Further X-ray and paramagnetic resonance data was available for acetates of Ni^{++} Co^{++} and Mn^{++} Therefore we undertook a detailed investigation of paramagnetism of the crystals of cupric acetate monohydrate and tetrahydrated cupric formate Nickel acetate Cobalt acetate and manganous acetate.

Vaokurov[10] reported the use of magnetic susceptibilities for the purpose of chemical analysis of Siderite a naturally occuring crystal of Fe^{++} ion ($FeCO_3$) X-ray data of the mineral by Wyckoff[11] indicated the existence of a trigonal crystal field acting on Fe^{++} ion in siderite We have therefore included it also in our programme.

Elliot and Stevens[12] have theoretically worked out the susceptibilities of cerium ethyl sulphate taking the crystal field to be of trigonal symmetry They have extended their theory (utilising a trigonal field) for other rare earth ethyl sulphates also. Therefore if one studies cerium sulphate a comparison of results with those for ethyl sulphate might throw some light on the nature of crystal field. This is the advantage of including cerium sulphate octahydrate crystals in our project.

Experimental

Experimental investigations have been carried out by usual methods. For magnetic anisotropies of crystals in various planes the well known method of Krishnan and Banerji[13] has been used while a new magnetic microbalance developed by Neogy and Lal[14] was made available for principal susceptibility measurements. Measurements down to liquid oxygen temperature were carried out using a cryostat as devised by Bose.[15] High temperatures were produced by a cylindrical electric heater

Discussions

Detailed experimental data will be published else where Here we proceed to outline briefly the interesting findings

(1) In case of cupric formate tetrahydrate the anisotropy in 10^{-6} of C G S. units, in (001) plane is only 37 2 while in planes normal to a and b axes the value of anisotropy in same units are 348 3 and 400 7 respectively Thus we find that c plane is a symmetrical plane. This finding of ours is in very good agreement with X-ray analysis findings.

(2) The mean moment values of Cu^{++} ion in cupric acetate monohydrate and cupric formate tetrahydrate are anomalously low At 300° K square of the mean Bohr magneto umber for the cupric acetate monohydrate is 2·017 and for cupric formate tetrahydrate it is 2·660, while the corresponding value in Tutton salts is 3·60

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(ii) Measurements with cerium ethyl sulphate octahydrate show that the crystals have an anisotropy of the order of $Pr_2(SO_4)_3 \cdot 8H_2O$ and is much different from Cerium ammonium sulphate tetrahydrate. Susceptibility value of cerium sulphate octahydrate is of the same order as for cerium magnesium nitrate with 24 water molecules and is much higher than the corresponding value for $Ce(NH_4)(SO_4) \cdot 4H_2O$

ACKNOWLEDGEMENTS

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SERUM AGAR DOUBLE DIFFUSION STUDIES ON STREPTOCOCCUS AGALACTIAE TOGETHER WITH AN ATTEMPT TO SERO TYPE STRAINS BY INDIRECT BACTERIAL HAEMAGGLUTINATION HAEMOLYSIS AND CORRESPONDING INHIBITION TESTS

B. D. BHAMBANI†

Sero-typing of group B streptococci by conventional serological methods presents certain difficulties. An attempt has been made in the work presented to sero-type group-B streptococci of bovine mastitis origin by employing Ouchterlony gel-diffusion, haemagglutination, haemolysis and respective inhibition tests

Preliminary studies on gel-diffusion revealed that the temperature of incubation *i.e.*, 20°C and the distance of 10 mm. between the reactant wells have marked influence in the early appearance of precipitin lines in plates. A slide test was developed for typing of *Streptococcus agalactiae* which proved superior in sensitivity resolving power Besides the test could be performed with very little quantity of reactants and the results could be read in a much shorter time.

Two types of antigens *viz.* (1) autoclave extracts and (2) acid extracts were employed in carrying out the antigenic analysis of fifteen different strains. The results of the test showed the antigenic complexity of these strains which contained both group specific and type specific components. In some strains the presence of atleast seven type specific components in addition to two group specific components were revealed

It was possible to classify *Str agalactiae* strains by employing the Ouchterlony technique. Of the 15 strains of streptococci thus examined, 8 strains fell under Lancefield type II 4 conformed to type III and 2 to type Ia. One type Ib strain obtained from Lancefield showed only group specificity in common with the rest of the 14 strains. As no antiserum against this strain was prepared, its exact type specificity could not be determined

This limited study has indicated that in India also there is a likelihood of a higher incidence of Lancefield type II strains to 77 7%. Streptococci of Lancefield type III have also been recorded in this country

From the scrutiny of the appearance of bands from a mixed precipitin system it was observed that the time factor had an important role. First of

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† Present Address B. D. Bhambani, M. V. Sc., Research Officer (Virology & Bact.) U. P. College of Veterinary Science & Animal Husbandry Mathura.

all a group specific and a type specific band made their appearance within 36 hours With incubation for a further period of 12 hours, another group specific band developed later with prolonged incubation the type specific band gave a diffused appearance possibly due to super-imposition of various type specific components which separated off and showed themselves as discrete bands with still further ageing The elution of different components was much better atleast quantitatively with acid extracts than that in the case of autoclaved extracts in which the lines were very thin though clear and sharp.

Live cell suspension in buffered saline left in the refrigerator for 2 days and 1 month when tested showed the presence of only one group-specific component. This could perhaps be explained by the fact that this fraction formed the surface antigen of the organism. With 18 hours glucose broth cultures of different strains used as antigens only strain 090 (Lancefield type Ia) gave a visible precipitin line representing group-specific component and none of the other group-B strains showed any line This indicated that perhaps in the case of 090 strain the surface group specific component was in somewhat loose attachment

The group specific antibody started appearing in the peripheral circulation of the rabbits under immunisation by about 20th day but the type specific antibodies could be detected only after a prolonged course of hyper immunization with living organisms It was seen that with additional courses of inoculations the successive specimens of sera showed increasing number of precipitin lines, indicating that antibodies to different antigenic fractions appeared at different times and that perhaps a reinforced antigenic stimulus was required Further some rabbit and strain variations were noticed.

Staining with azocarmine of precipitin reaction on gel diffusion slides indicated the usefulness of this method in preserving the reaction for period of atleast two years and perhaps indefinitely

It could not be possible to characterise group-B streptococci into clear cut sero-types by the keogh haemagglutination haemolysis and haemagglutination inhibition tests. Lancefield representative group-B strains gave haemagglutination and haemolysis titers which were comparatively lower than those obtained with strains isolated from clinical cases of bovine mastitis in India.

It may be added that the strain Ladly of streptococci (Lancefield type II) included in the present study evoked higher precipitating antibody content in immunised rabbits. It gave maximum number of reacting components in gel diffusion test. The possibility of using this strain for immunological purposes should be explored

INCIDENCE OF *FUSARIUM* WILT OF GRAM (*CICER ARIETINUM* L) IN OILCAKES AMENDED SOILS*

S K CHAUHAN
Botany Department Agra College Agra

Use of organic and inorganic manures for control of diseases caused by soil fungi has been suggested by various workers King and Loomis (1926) reported that heavy application of manures or other organic materials consistently reduced the cotton root rot. Hooker Walker and Link (1945) have observed the controlling effect of two mustard oils for the Club-root disease of crucifers. Chowdhury (1946) suggested that mustard oilcake can greatly reduce the incidence of *Sclerotial* wilt of *pan* The present investigation deals with the influence of certain oilcakes on the incidence of gram wilt caused by *Fusarium orthoceras* App and Wr var *ciceri* Padwick. The oilcakes contain N P and K (th three critical elements) while the mustard oilcake contains S in addition.

METHODS AND MATERIALS

Three kind of oilcakes (i) groundnut oilcake ( ) til oilcake and (iii) mustard oilcake were used to amend the soil Twelve replications were maintained throughout the course of the experiment for each treatment and its control.

8 lbs. of garden soil was filled in each pot (9"×12") of the experimental terra. Half pound of finely grounded oilcake powder was uniformly mixed with the soil in each treatment. One set of pots contained only garden soil which indicated wilt incidence in normal soil

The soil was inoculated in the usual way The pathogen was mixed with the soil of each pot in all the d fferent treatments and also in the unamended soil. A control where the soil was not infested with the pathogen was separately maintained for each treatment. Three days after inoculating the soil five seeds of gram (variety T 87) were sown in each pot Throughout the period of experiment the optimum conditions of disease development were provided as worked out by the author (1959)

When the plants began to wilt the number affected each day was recorded separately for each treatment. In the control experiment none of the plants showed any sign of wilting in any of th treatments under observation. The figures for total mortality were analysed statistically and the progressive rate of mortality plotted on a graph fo the different treatments

The different oilcakes were analysed for the percentage values of nitrogen, phosphorus potash and sulphur following standard chemical methods.

* A part of pproved Ph. D thesis of Agra University

Results

The data of oilcakes analysis and the series dealing with the effect of various oilcakes are presented below

TABLE 1

Showing percentage value of each

Name of oilcake	Nitrogen	Phosphorus	Potash	Sulphur
Groundnut oilcake	7 32	1.54	1.31	——
Til oilcake	6 22	2 00	1 21	——
Mustard oilcake	5 20	1 83	1 21	

Phosphorus in the form of Phosphoric acid.
* Present in sufficient quantities.

The above table indicates that the percentage values of nitrogen phosphorus and pota h is not the same in each oilcake under study Fur ther it has been shown that the mustard oilcake has sufficient quantity of sulphur in addition to N P and K

TABLE 2

Analysis of variance for total Mortality

Treatment due to	D F	S S	M S S	F
Oilcakes	3	10750	3583 33	79 62
Error	20	900	45 00	
	23			

Experiment significant at 5 level. C. D. =3.995

It is th s clear that the effect of individual factor is significant indic ting the reliability of such experir ent

TABLE 3

Showing percentage mortality in d fferent treatments

Soil non-amended	S l amended with groundn t oilcal	Soil mended with til oilcal	S l amended with mus ard oilcal
63 33 ± 6 31	70 00 ± 03	18 33 ± 01	8 33 ± 1 1

The above data indicate that by amending the soil with oilcakes there is a significant reduction in percentage mortality. The order of reduction being soil 63·33 > groundnut oilcake 20·00 > til oilcake 18·33 > mustard oilcake 8·33.

There is, however no significant difference between percentage mortality in til oilcake and groundnut oilcake the figures being 18.33 and 20·00 respectively (C. D 3·995). In mustard oilcake it is significantly lower i. e. 8·33. The different treatments can be grouped as follows —

Soil untreated	Soil with mustard oilcake	Soil with til oilcake	Soil with groundnut oilcake
63·33	8·33	18·33	20·00

The maximum rate of mortality occurs after fifteen days for a period of one month, in all the treatments under trial. In mustard oilcake amended soil, the disease appears only during this period and afterwards becomes steady indicating perhaps a very suitable period of disease incidence. The tempo of disease development is almost the same in groundnut oilcake and til oilcake treated series. A precise idea of the tempo of disease development is indicated in Graph.

Conclusion

Generous application of fertilizers is undoubtedly an effective way of reducing the proportion of crop loss caused by root diseases. According to Garrett (1942) the proportional loss occasioned by the take-all disease of wheat is greatly aggrevated by poverty of the soil in one or more of major nutrients. A generous supply of plant nutrients probably increases plant resistance chiefly by promoting a more rapid production of new crown of roots to those destroyed by the disease. Since the work of Smith and Walker (1911) the scope of investigations on the effect of nutrients upon development of diseases has greatly enlarged. It has been pointed out by Dick and Tisdale (1938) that when nitrogen and potash are jointly applied, the incidence of wilt disease is very considerably reduced. Addition of nitrogen alone increases vascular wilts (Clayton 1923, Fisher 1935) while the effect of potash is beneficial (Young 1935). Oilcakes under trial in the present investigation have both nitrogen and potash and they reduce the incidence of the wilt disease of gram. By amending the soil with mustard oilcake the disease is reduced more due to the action of sulphur which is an extra constituent in this oilcake. Further the proportion of nitrogen in mustard oilcake is less than in other oilcakes used in the experiment.

Summary

Wilt of gram (*Cicer arietinum* L.) caused by *Fusarium orthoceras* var. *ciceri* has been studied in relation to different oilcakes amendments. Soil was

amended by three kinds of oilcakes viz. groundnut oilcake til oilcake, and mustard oilcake. It was noticed that by the application of oilcakes there is a significant reduction in the percentage mortality Mustard oilcake proved to be the best in controlling the disease. The remaining two oilcakes proved to be of casual value, while in the untreated soil the disease was quite high nearly 64% The data were analysed statistically

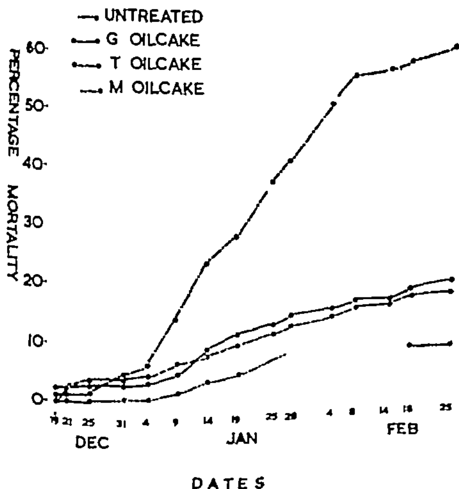
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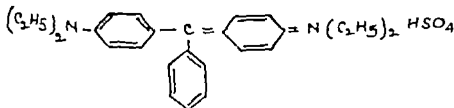
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PHYSICO CHEMICAL STUDIES ON BRILLIANT GREEN

SARVAGYA S KATIYAR
Chemistry Department, Indian Institute of Technology
Agricultural Gardens Post Box No 939 Kanpur

Studies on the reactions responsible for the unstable nature of the dyes of triphenylmethane series have been the subject of recent investigations and attracted the attention of many workers on account of their marked industrial importance as dyeing agents. Brilliant green (B. G) chemically known as 44'bis diethylamino triphenyl methyl sulphate



which is one of the important dyes of the triphenylmethane series is of characteristic green colour and shows absorption maximum at λ=625 426 and 319 mμ in the visible and ultraviolet regions of the spectrum and has been employed as not only a dyeing agent but also for analytical purposes, for estimation of Au Tl etc. The characteristic colour of B. G decreases with time in acid and alkaline medium. Kinetic study of the nature and mechanism of the reaction (s) responsible therefor formed the subject matter of this dissertation.

In part I of the Thesis are presented detailed investigations on the kinetics of decomposition of B G in alkaline medium while Part II reports data on the same in acid medium. Since the reactions occurring in these solutions are of different nature (as indicated by the results) the presentation of the results in two separat parts has been necessitated.

The absorption spectrum of B. G was investigated over a wide range of concentrations and at different pH values where the kinetic studies were sought to be investigated Careful control of the experimental conditions was an essential requisite for these studies on ccount of th incipient decomposition and the associated decrease h racteristi extinction coefficient and also due to adsorption of th dye on glass surface Data were presented to show that Beer s law wa applicable under the experimental conditions selected f kinetic investigation this ppeared to be a prerequisite for the successful completion of the investigations and valid analysis of the dat obtain-

This is an abstract of the thesis submitted and pproved for the degree of Doctor of Philosophy of the Agra University in 1962.

ed therein The results presented in chapter 2 showed that the reaction responsible for decolourisation or fading of the colour of the B. G solution in alkaline medium was bimolecular and second order in nature the velocity of the reaction was markedly dependent upon the concentration of OH^- The apparent rate constants (k) of the reaction under conditions of known and excess of $[OH^-]$ were computed and the real bimolecular constants (k') were computed ($k' = \frac{k}{[OH^-]}$) The reaction appeared very fast at pH = 11·20 the deep green coloured solution (corresponding to O D 0 300) became colourless within 35 m nutes ($t_{\frac{1}{2}}$ = 4 8 min) So long as the alkali or $[OH^-]$ was present the decomposed solution remained colourless when OH^- was removed from the sphere of reaction by addition of calculated quantity of an acid the colourless reaction system started gradually acquiring the characteristic green colour with absorption maxima at the wavelengths as of original B. G solution (see also Fig 2 VIII) These studies proved that the reaction was a reversible one and acquired unidirectional nature in excess concentration of OH^- The influence on the velocity constant of the reaction of various factors such as (a) concentration of B G and OH^- (b) temperature (c) ionic strength and (d) dielectric constant of the system was investigated While the studies on (a) proved the bimolecular nature of the reaction those on (b) gave the energy of acti ation and other thermodynamic properties of the reaction (chapter 4) The data on (c) and (d) when analysed on Bronsted-Christiansen-Scatchard equation gave a remarkably interesting result about the nature of the reactant, in add tion to OH^- the species of B G which involved in the reaction was positively charged in nature (chapter 3) These studies led the author to suggest for the first time in the field of research the followi g mechanism (chapter 6)

(i) B G first ionises to yield a positive ion (fast)

(ii) The positive ion of B G resonates with another species carrying a pos ti e charge on central carbon atom (fast)

(iii) Th ion of B. G with positive charge on central carbon reacts w th OH^- ion to f rm carbinol (rate determining step)

Part II de ls with as pointed out earlier the kinetic studies on the react on of B G in acid s lutions

The ch aracteris rate constants of this reaction were markedly distinct from those bta ed for the eaction in alkaline solution These were computed for var us concen rations of B G and H^+ As in the case of alkaline ol tions the dec mpositio f B G in acid s luti n was reversible in nature The k etic studies were therefore carried out in excess of con centration of H^+ ion wh h appeared to be one of the reactants in the bimolecular rea tio n From the studies on the influence of temperature of reaction ΔS and their thermodynamic con tants were computed (chapter 10) While $E^{\neq}$ and $\Delta S^{\neq}$ were 1? ?? kcals and −?·1 cal deg

mole for the decomposition of B. G in alkaline solutions the same were 11·65 kcals and −24·36 cal/deg mole respectively in acid solutions The data on the effect of ionic strength and dielectric constant on velocity constant of the reaction in acid solutions (chapters 8 and 9) showed that unlike in alkaline solutions the reaction in acid solutions involved two species with similar unit charge thereon. Since the H^+ is one of the reactants, it is deduced that the other is the positively charged ion of B G The following mechanism was suggested

(i) B. G first ionises to yield a positive ion (fast)

(ii) Due to resonance another cation of B G carrying a positive charge on central carbon atom is obtained (fast)

(iii) Cation of B. G with a positive charge on central carbon atom reacts with H^+ ion to give acid salt (rate determining step)

The data presented in this dissertation show clearly for the first time in this field of research that the same ionised species of B G on reaction with H^+ and OH^- ion gives different reaction products for which evidence has been adduced. The mechanisms for the reactions were put forward which elucidated all the observed facts.

The results on the variation of velocity constant with the composition of the acetone-water mixtures used as solvent showed that 30% acetone-water mixture could be safely used for the colorimetric estimation of Tl and Au which has been tested and found extremely suitable.

At the end of the Thesis is presented an Appendix which reports polarographic investigations on the behaviour of B. G at dropping mercury electrode. These studies though unconnected with the main work of the dissertation were the result of academic interest of the author in examining the recent theory of irreversible reaction systems Simple cations like Cd^{++} Pb^{++} etc. are reversibly reduced at d. m. e. and the polarographic data thereof are amenable for analysis by Heyrovsky Ilkovic equation (similar to Nernst equation of electrochemical reactions) Complex organic substances undergo reduction at d. m. e and the corresponding data are disobeyed by the above equations for reversible systems. For elucidation of the irreversible reduction processes, various mechanisms have been put forward Of these, the one developed by Delahay assumes the occurrence of a time process preceding or following the reduction process. B. G gave at d. m. e two reduction waves both of which were irreversible in nature, which was proved by independent and varied experiments Data on the diffusion coefficient employing McBain and Dawson cell were computed as these were required for the application of Delahay's theory The rate constants of the reaction preceding the electrochemical reduction process were computed. A mechanism for the overall reduction process was suggested.

STUDIES OF THE ENZYMIC REACTIONS OF UREASE*

VISHNU CHANDRA†

Assistant Professor of Chemistry B. R. College Agra

SUMMARY

Urease is an enzyme of special interest, because it was the first enzyme to be obtained in the crystalline form.[1] The present investigation was undertaken to study the following aspects of its activity (1) To investigate the decomposition of urea under varied experimental conditions (2) To find out the influence and mechanism of action of Ag^{+} Cu^{++} Au^{+++} Pb^{++} Sn^{++} and Sn^{++++} ions on the decomposition of urea by urease and (3) To investigate the possible role of urease in the protein metabolism of plants which contain this enzyme in appreciable quantities.

Urease activity is usually determined by allowing a known quantity of urease to react with excess of urea in presence of a buffer solution of pH 7 for a definite interval of time. The reaction is then stopped by adding acid and the ammonia produced is aerated off and estimated either by nesslerisation or by titration. In the present investigation urea urease buffer etc., are mixed together and 5 ml of the reaction mixture are taken out and titrated against N/50 sulphuric acid using methyl red indicator. Suitable controls are also run simultaneously.

The work has been divided into four chapters. The first chapter deals with the decomposition of urea by urease under different experimental conditions. The decomposition of urea has been studied with commercial (B.D.H.) as well as soyabean urease, by using the above mentioned method of determining the urease activity. The presence of urease in soyabean (*Glycine soja*) was discovered as early as 1909 by Takeuchi,[2] but since then not much work has been done to investigate the reaction kinetics of the urease present in soyabean in its natural environment. Although it has usually been believed by biochemists that urease obtained from different sources is the same, the variations in the isoelectric point of the urease obtained from different sources[3,4] and the difference in the optimum pH of their reactivity[5] leave doubt whether the various enzyme samples obtained from the different tissues may not be different proteins having a common urease activity. There is almost complete lack of knowledge regarding the role of urease in plants. To study the function of urease in the tissues a thorough investigation of the proteins with urease activity in their natural environments, as well as a

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† Present address Chemistry Department, Gorakhpur University Gorakhpur

study of the possible functions they may be playing in plant and animal tissues with their urease activity becomes much more essential. It is with this end in view that the present investigation was undertaken.

The investigation of urea decomposition by varying quantities of aqueous soyabean extract has been undertaken and it has been observed that though soyabean contains urease in sufficiently active form the high concentration of the extract does not increase the decomposition of urea proportionately. This seems to indicate the presence of some urease inhibitor in the extract. It appears that the ammonia set free by the decomposition of urea is utilised in the conversion of organic acids formed by the extract into amino acids which subsequently form peptides.

In studying the decomposition of urea by commercial urease it was observed that the decomposition of urea increased by increasing the concentration of the enzyme to a certain extent, beyond which the increased rate of urea decomposition is not observed though the mixture contains enough undecomposed urea. The influence of substrate concentrations on the urease action has also been investigated. It has been found that the decomposition goes on increasing by increasing the concentration of urea.

The influence of pH on the decomposition of urea by commercial as well as soyabean urease has also been investigated. It has been observed that although urease is quite active in the pH range 6·8—10·0 the optimum pH for phosphate buffer is 7·6.

The second chapter deals with the effect of Ag^{+}, Cu^{++}, Au^{+++}, Pb^{++}, Sn^{++} and Sn^{++++} ions on the decomposition of urea by urease. Silver, copper and gold belong to the I B group of the periodic table and the influence of their ions has been determined at pH 6·8, 7·8 and 8·8, by using solutions of silver nitrate, copper sulphate and gold chloride. All the three have been found to be inhibitors of urease. Silver ions have been found to be the strongest and gold ions the mildest inhibitors. The experiments performed with these ions show that the inhibition increases with the increase of concentration of the metallic ions and within a particular limit of the metallic ion concentration, the inhibition is proportional to the metallic ion concentration. The decrease in the inhibitory influence with an increase in the valency of these ions may be explained by supposing that a divalent ion (like Cu^{++}) may form a complex with two molecules of urease and a trivalent ion (like Au^{+++}) may form a complex with three molecules of urease thereby leading to the formation of ladder like and tripod like structures. These structures will offer steric hindrance for the further deactivation of active centres of the urease molecule and obviously this steric hindrance will be greater with tripod like structures than with ladder like structures, and the greater the steric hindrance the greater will be the number of ions required to deactivate a molecule of urease. Hence gold ions which are

tavalent, will be required in larger numbers to deactivate urease completely than copper ions which are divalent. In the case of silver ions which are monovalent, the question of this steric hidrance does not arise and hence they should be the strongest inhibitors and that has been found to be the case.

The influence of lead ions has been determined by using lead acetate solutions. Lead acetate acts as an inhibitor in the decomposition of urea by urease for some time after which, as if the enzyme has acclimatised and has adapted to it, the inhibition decreases and finally the metal ion activates the reaction. The inhibition is indicated as either poor requirement of the standard acid solution in the titration of free ammonia or as total inactivity of the enzyme when no urea is decomposed for sometime thus indicating a lag period in the enzymic activity

In this series where influence of lead acetate on the decomposition of urea by urease has been studied, a case similar to biological adaptability and acclimatisation is observed in a purely chemical mixture. Whatever may be the actual mechanism of decrease in the inhibitory influence of lead ions on urease with increase of time and finally resulting in activation or whatever may be the reason for lack of activity for sometime in presence of lead ions and then activation of the enzyme these observations are very much similar to biological adaptability and biological acclimatisation respectively Observance of such a phenomenon in a mixture of non-living substances merely by an enzyme is of great academic interest in the study of molecule evolution leading to the formation of pro-protoplasm or protoplasm in the pre-biological era of the earth.

The influence of Sn^{++} and Sn^{++++} ions on urease activity has been determined by employing solutions of stannous chloride and stannic sulphate respectively It has been observed that Sn^{++} ions act as strong activators for urease, and pH 6.8 has been found to be optimum for this activation The activation by Sn^{++} increases by increasing its concentration upto a certain limit and then sharply declines by further ncreasing its concentration. Sn^{++++} ions have also been found to be strong activators of urease.

The third chapter deals with an attempt to investigate the possible role of urease in protein metabolism of plants which contain it in appreciable quantities. Urease has been found to be absolutely specific, although Shaw and Kistiakowsky[7] have stated that biuret is hydrolysed by crystalline urease. The only reaction indicated by the enzyme is the decomposition of urea with the liberation of ammonia. It is not yet understood as to what function the enzyme plays in the biological tissues where t is found because in many tissues where it is found no urea or free ammonia has been detected at any stage. The presence of high concentrations of urease in soyabean which is very rich in, protein content suggested the possibility of urease activity in

the protein synthesis or that it is in some way related to the protein metabolism of the bean tissue.

To study the possible role of this enzyme in tissues, boiled soyabean powder extract and boiled gram powder (*Cicer arietinum*) extract, separately as well as in presence of each other have been tried as substrates for urease. Gram powder does not contain any urease but was selected on account of its rich protein content. The solutions were boiled to inactivate all the enzymes present in these solutions so that any reaction which may occur may be attributed to the action of urease only. Suitable control experiments were also carried out to eliminate possible errors due to the presence of other substances present in such natural substrates as gram powder and soyabean powder. The amino acid content of the reaction mixtures was determined by titrations of the neutralised reaction mixtures against N/50 sodium hydroxide solution in presence of neutral formalin. The results of the experiments indicate that although there are some indications of proteolysis and peptide formation by urease it cannot be said conclusively that this is the case.

The fourth chapter deals with an investigation to determine if urease acts as a coenzyme for some proteolytic enzyme. To determine this, its influence on the proteolytic activity of papain has been investigated. An attempt has also been made to investigate as to how far amino acids influence the decomposition of urea by urease and glycine was chosen for this series of experiments.

The influence of urease on papain has been determined in presence of stannous chloride which acts as a strong activator of urease. It has been found that papain acts as a strong activator for the decomposition of urea by urease and its use also results in the increased formation of amino acids, i.e. proteolysis of proteins of the mixture also takes place simultaneously. The decomposition of urea as well as the proteolysis increase to a *certain* extent by increasing the concentration of papain. Both the reactions are then slowed down by a further increase in the concentration of the enzyme. The optimum pH for both the reactions has been found to be 6.8 although both the reaction occur *appreciably at pH 7.8* and *8.8* also. Though Zakowski[3] had observed that urease was not attacked by hydrocyanic acid activated papain at pH 7.0 and was markedly weakened at pH 4.4—5.5 his work was limited to a study of the proteolytic action of papain against the protein of the urease molecule and no investigation of the *combined influence* of urease and papain has been carried out. Experiments described here indicate that instead of proteolysis of urease by papain urease is activated by simple papain which has not been modified by any activator as hydrocyanic acid or hydrogen [illegible] This activation is not unidirectional but is equally shared by pa[illegible] cules as well as [illegible] far more proteolysis of the proteins of soyabean by [illegible] observed than with papain alone

The experiments with glycine were carried out with a view to study as to how far urea is essential in mixtures of urea urease stannous chloride, and glycine, which show increased ammonia formation and proteolysis of soya bean proteins. It has been found that urea is absolutely essential for increased formation of ammonia as well as for the formation of amino acids. The experiments performed in presence of urea indicate that glycine acts as a strong activator in the decomposition of urea by soyabean urease. The activation increases by increasing the amount of glycine.

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MORPHOLOGICAL STUDIES OF THE FLOWER OF MYRTACEAE WITH SPECIAL REFERENCE TO ITS VASCULATURE

Km. Ishwari Gupta†
School of Plant Morphology, Meerut College, Meerut

Summary

The present studies include 41 species of which 35 belong to Myrtaceae and five to Lecythidaceae.

It has been seen that the inflorescence in Myrtaceae presents a wide range of variation. Inflorescence in Lecythidaceae is racemose or cauliflorous. Modes of specialization of inflorescences are illustrated by schematic diagrams.

It has been observed that secretory cavities are of general occurrence in Myrtaceae, but are absent in Lecythidaceae.

Occurrence of tannins also follow the same rule as do the secretory cavities.

The sepal lobes are either free with a single vascular bundle or they are more or less fused to form Calyptra (*Psidium*) or operculum (*Eucalyptus*). In latter case they receive large number of vascular supply.

Petals in all except *Eucalyptus* are normal. In this genus they form an inner operculum with large amount of vascular supply.

The stamens are numerous one trace organs. They receive their vascular supply from a trunk vascular supply "the stamen fascicle trace". These traces are supposed to be produced by cohesion of stamens and their vascular traces.

The study of style and stigma reveals several variations which appear to indicate some trends of specialization in the gynoecium. The stylar canal or transmitting tissue is formed by the inward movement of some of the epidermal or subepidermal cells of the placental region. The lobes of the stylar canal are equal to the number of the carpels present.

The stigma is carinal and rarely (*Leptospermum*) commissural. In either case it has been observed that secondary marginal bundles are more prominent.

A tendency towards gynobasic style has been observed in *Callistemon* and *Melaleuca*.

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† Present address: Head of Botany Deptt., Mahila College, Lucknow.

The placentation is interpreted to be axile apparently but parietal anatomically. Evidence for such a suggestion has been obtained from the course and the behaviour of vascular bundles supplying the placentae and the ovules. It has been further suggested that axile is giving way to parietal placentation.

The ovary is inferior but rarely semi-inferior. No conclusive evidence has been obtained regarding the nature of the inferior ovary.

Anatomy of the operculum shows that it has different origin in different group of species. Probable origin of operculum has been traced from calyptrate petals present in *Syzygium* species.

Circumscissile dehiscence of the operculum is brought about by breaking, disruption and separation of cells at the zone of dehiscence at the time of anthesis.

The type of node present in the six members studied shows that the node is always unilacunar which has been considered to be derived from the primitive two-trace unilacunar node.

From the summation of the evidence it has been concluded that the three genera *Barringtonia*, *Careya* and *Couroupita* should be placed in a separate family Lecythidaceae.

STUDIES IN THE PATHOGENICITY OF CERTAIN PARASITIC FUNGI WITH SPECIAL REFERENCE TO THEIR PECTIC ENZYME SYSTEM

M N Gupta

Associate Professor Botany Department Agra College Agra.

In the present investigations three parasitic fungi namely *Rhizoctonia solani* causing root rot of linseed and other hosts *Fusarium orthoceras* f *ciceri* causing wilt of gram and *Penicillium expansum* responsible for the soft rot diseases of many fruits were studied in detail for the production of pectic enzymes *in vitro* The properties of these enzymes and their possible role in the pathogenicity were also investigated. Following are the more important findings

1 Although a number of natural media were used, none of them was found to be suitable for the active enzyme secretion for any of the three fungi. However many of them supported very good growth of the fungi.

2. *F orthoceras* f *ciceri* and *P expansum* secreted active enzymes on media which contained pectic substances while *R solani* was able to secrete these enzymes on a large number of media even in the absence of pectic substances

3 *R. solani* and *P expansum* showed no special liking for any carbon source as they could secrete active enzymes with a number of carbon sources *F orthoceras* f. *ciceri*, however preferred cellobiose for active enzyme secretion

4 Though the three pathogens were able to utilize a good number of nitrogen sources both organic and inorganic yet the best results were obtained with ammonium nitrogen.

5. PP PG and PE enzymes behaved differently when subjected to the variations of temperature pH, dilution and purification. This clearly indicates that PP PG and PE are separate enzymes.

6 PP PG and PE of the three fungi were found to be highly heat resistant as they were not completely deactivated on autoclaving at 120°C for 20 minutes. This was more so with PG enzyme. However recovery of the enzyme activity at 70°C, after it had shown a sharp fall at 60°C, was observed in the case of *R. solani* and *F orthoceras* f *ciceri*. No such recovery was observed in the case of *P expansum*.

7 PP enzyme showed maximum activity at higher temperatures as compared to PG and PE.

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8 Purification by precipitation with alcohol and acetone has been found to be much better than dialysis as in the latter case the enzyme lost much of its activity

9 PG and PE of the three pathogens were most active at pH 6.2 while the optimum pH for the activity of PP was in the neighbourhood of 9.0

10 Enzyme preparations from the three sources could be successfully stored as crude culture filtrates at 0°C under toluene for 30 days without much loss in their activity When stored as dry precipitate they lost their activity much more rapidly

11 Enzyme preparations obtained from *P. expansum* which contained very little PE but active PG showed very rapid breakdown of pectates as compared to pectin while the enzyme preparations from *R. solani* and *F. orthoceras f. ciceri* affected both the compounds with equal rapidity In the latter two fungi PE was more active than the former pathogen. This has led to the conclusion that either DP is comparatively less active in *P. expansum* or this difference is due to the very poor activity of the PE enzyme

12 Studies on the specific rotation of mixtures in which pectic enzymes were reacting with pectic substances and chromatographic studies of the breakdown products of pectin and sodiumpolypectate solutions have indicated the possibility of the presence of one or more unidentified enzymes which further break down the monogalacturonic acid chain

13 There exists no relation between the ability of an organism to parasitise a tissue and to secrete the macerating enzymes in the decoction of that tissue.

14 There are evidences that plant tissues contain certain inhibitors which decrease the enzyme activity when both are mixed *in vitro*.

15 It has been proved that pectic enzymes play a definite and important role in the development of wilt symptoms They bring about the dissolution of the pectic substances of the cell wall which accumulate and plug the vessel

16 Not only PG and PE but also PP has been shown to be important in pathogenesis as it brings about the disorganisation of host tissues.

N. B. The details of the investigations are under publication in various journals

STUDIES ON SOME MYCOTIC INFECTIONS OF DOMESTIC ANIMALS AND POULTRY

RAM PRATAP TEWARI

Department of Pathology and Bacteriology

U P College of Veterinary Science & Animal Husbandry Mathura

Recognising the importance of mycotic infections in this era of increasing antibiotic therapy the studies on some mycotic infections of domestic animals and poultry has been undertaken In a general way an attempt was made to explore the possibilities of common prevailing fungus diseases in northern part of country and the infections studied were dermatomycoses candidiasis, *Trichosporon* infection rhinosporidiosis and phycomycosis. A total of 5,596 animals represented by 2268 cattle 1874 buffaloes, 172 dogs, 112 horses, 60 goats 5 sheep 230 pigs 875 poultry 3 human beings and 18 soil samples were examined during the course of this study

The primary isolation of these dermatophytes was attempted on four different media starting from Sabouraud agar supplemented with penicillin and streptomycin but a complimentary pair of cycloheximide and Morena desoxycholate media appeared to be more satisfactory for the purpose. The Riddell's slide culture technique used in this study also appeared to be very helpful in the identification of ringworm fungi.

One hundred and nine strains of ringworm fungi represented by five species of dermatophytes *M gypseum* 9 *T mentagrophytes* (both varieties) 42 *T terrestre* 43 *T rubrum* 13 and *T violaceum* 2 were isolated from clinical cases of ringworm, from cattle, dog horse poultry and man.

Out of these the isolation of *T mentagrophytes* var *granulare* *T terrestre* and *T violaceum* from cattle *M gypseum* and *T mentagrophytes* var *granulare* from horse are probably the new host records for India, while *T mentagrophytes* var *interdigitale* from dogs *M gypseum* and *T mentagrophytes* var granulare from poultry do (downy) from cattle and T mentagrophytes var granulare from poultry do not appear to have been recorded on any previous occasion

The over all incidence of ringworm in cattle came to 2·9 per cent while the corresponding figures for other animals were, dog 9·3 per cent horse 8·0 per cent and poultry 1 9 per cent Age appears to have a marked effect on the incidence of ringworm in cattle calves under one year being more susceptible than mature cattle

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It is interesting to note that the same dermatophytes *T. verrucosum* and *T. mentagrophytes* var. *granulare* were isolated from cattle and poultry respectively as well as from their attendants the public health implications of which has been stressed.

Three strains of *Sepedonium* species one from a dog and other two from horses were isolated from skin lesions of these animals. The possible significance of which could not be ascertained as no previous record of such an infection is available. Besides these two strains of *M. gypseum* were isolated by hair bait technique from soil samples collected from different places.

A total of 215 *Candida* strains represented by *C. albicans* 206, *C. tropicalis* 3 and *C. rugosa* 6 were isolated from normal as well as from the crops showing gross lesions resembling to turkish towel. C. rugosa has probably been recorded for the first time in poultry.

A markedly higher incidence of *Candida* in the crops of adult birds above four weeks (56·6 per cent) as compared to chicks under four weeks (14·6 per cent) was observed.

In cattle *Candida* species were isolated from nasal passages of 61 (51·0 per cent) out of 111 nasal granuloma cases and 7 (6·6 per cent) out of 106 apparently normal animals examined culturally. These 60 strains of *Candida* were represented by *C. tropicalis* 20, *C. albicans* 5, *C. guilliermondii* 1 and *Candida* species 33.

Simultaneously two different media viz. corn meal agar with tween-80 (C. M. T.) and sodium taurocholate agar (S. T. A.) were tested for the production of chlamydospores by 250 *Candida* strains with an idea of comparing their utility. It is felt that S. T. A. inspite of its simpler composition is equally comparable with C. M. T. if not a little superior as for chlamydospore production is concerned. All the above *Candida* strains were also tested on Pagano-Levin medium and it appeared to be a good adjunct in the identification of *C. albicans*.

Fiftyone strains of *T. cutaneum* 24 from crops of poultry and 37 from nasal passages of cattle were isolated.

No case of rhinosporidiosis could be detected during the course of this study.

A case of phycomycosis in a buffalo calf of one and a half year of age which died after a brief clinical history of severe diarrhoea has been described.

The necessity of carrying out an extensive and well organised studies in the field of veterinary mycology and its epidemiological and public health importance in this country is stressed.

STUDIES ON THE PATHOLOGY OF BOVINE CARDIOVASCULAR SYSTEM*

KUSAL PAL SINGH
Department of Pathology & Bacteriology
U P College of Veterinary Science & Animal Husbandry Mathura U P (India)

Cardiovascular diseases and cancer have long been recognised as the most important problems in the field of medical sciences This awareness has led not only to the extensive studies and intensified research activities on the problems in the whole world but also the attempts have been made to study the condition experimentally in domestic and laboratory animals. The present investigation was undertaken to study the pathological involvements of the cardiovascular system of cattle and buffaloes and to compare the lesions in these animals with some of the similar lesions described in medical pathology

During this study 2306 animals including 134 autopsied animals in the Department of Pathology and Bacteriology and 2096 buffaloes slaughtered in various abattoirs of Uttar Pradesh were examined grossly The lesions were discernable grossly in 323 hearts and 711 aorta of the above animals Histopathological studies were conducted on 183 hearts and 243 aorta, out of which bloodvessels of 46 cases were also examined histochemically for fat and cholesterol. The deparaffinised sections of 5-7 microns thick were stained by routine haematoxylin and eosin method alongwith the special staining wherever necessary such as for the demonstration of microorganisms calcium salts, elastic tissue lipid cholesterol etc.

The lesions of lymphosarcoma involving the pericardium and epicardium of 217 (10·3%) cases among the 2096 slaughtered buffaloes were observed The involvement of the lymph nodes was seen in most of the cases alongwith the lesions in lungs liver spleen and the adventitia of the pulmonary artery in some cases Its higher occurrence in slaughtered buffaloes and the absence of these lesions in 80 young buffalo calves of 1 2 years in age sacrificed in the Surgery Department and in the 134 autopsies in the Pathology and Bacteriology Department are very striking

Bluish nodules at the valves of the heart were noted in 23 animals which histopathologically consisted of highly dilated blood vessels with lymphocytic infiltration and light proliferation of connective tissue around them. Such lesions are not frequently reported in the literature.

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Pericardial adhesions were noted in 68 cases which were in the nature of chronic non-suppurative inflammation of the serous membranes. The infestation of sarcocysts was histopathologically observed in 115 (62 5%) cases and purkinje fibres of the heart were affected in 23 cases Single cases of echinococcus cyst infestation of the heart and an unidentified parasite in the subpleural fat in slaughtered buffaloes were also recorded

16 cases of traumatic pericarditis and one case of tuberculous pericarditis were studied in buffaloes.

The aorta was found to be infested with *Onchocerca armillata* in 224 (9 7%) cases. The parasite was found to be doing damage to the aortic wall specially the media over which the elasticity of the aorta depends. Extensively tunnelling on the intimal surface thickening of the wall, aneurysm and nodules on th intima and occasionally on the adventitia were grossly observed. Histopathologically the parasites on the adventitia caused necrosis intense infiltrations of macrophages with foamy cytoplasm, lymphocyte and giant cells at the media in addition suppuration in a few cases was observed. On the intima nodular buldging was observed in some cases with parasites causing protrusion even to the formation of the nodules and papilla like structures Histochemical evidence for the presence of fat and cholesterol was found in the intima media and in the degenerated area around the onchocerca lesions, however the characteristic lesions of atherosclerosis or atheroma described in the medical pathology were neither grossly nor histopathologically observed in 2306 cases of this study

The calcification of the aorta grossly ranging from minute areas to the extensive plaque formations were observed in 24 cattle and buffaloes without onchocercosis. Histopathologically calcification was still more common and was seen in 77 out of 242 cases examined

Linear roughening of the intimal surface at the abdominal aorta was grossly noted in 340 (14 7) cases which was well associated with aneurysm in som ca es These rough surfaces microscopically showed the degeneration of the elastic tissue of the media and intima duplication dis ruption and f ys ng of the tunica elastica interna and fine granular deposition of calcium salt In som of the lesions the presence of fat and cholesterol was demonstr te l Pinpoint white raised foci diffusely scattered on the intimal surfac were grossly observed in 119 (5 1) ca es which were made up of exten i e d sorganisation of the elastic t ssue with deposits of the calcium salts

The other c ndit ns observed during the study were aneurysm of the aorta in 103 an l f mesenteric artery in 3 cases medial hypertrophy of the smaller arteries in 10 e associated with inflammatory involvement thrombosis of the smaller arteries f the heart lymphnodes spleen and vasavasorum in 10

cases; emboli in smaller vessels and lymphatics in 8 cases and focal intimal thickening of the coronary vessels in 14 cases The implications of various types of pathological involvements of bovine cardiovascular system are discussed and the necessity of carrying out further work is stressed.

STUDY OF ACTION OF LIGHT ON AMINO ACIDS IN PRESENCE OR ABSENCE OF ENERGY MATERIALS UNDER ASEPTIC CONDITIONS*

HARI DATT PATHAK
Th D S B Government College Naini Tal

The effect of exposure to sunlight and artificial light on pure amino acids separately and in combination with other amino acids with or without energy material, under aseptic conditions has been studied At the same time the action of sunlight and light of a 1000 watt bulb on aqueous solutions of pure amino acids with or without colloidal metallic oxides as possible photosensitizers had also been studied. In preliminary investigations it was observed that the ultra-violet component of sunlight was more effective in the peptide formation Therefore, the action of ultraviolet light on aqueous solution of tyrosine with or without colloidal Fe_2O V_2O and/or MoO_3 was also investigated. These investigations were carried out separately in quartz and pyrex vessels. The action of light on aqueous solutions of amino acids was observed under perfectly aseptic condition of the solutions to avoid any complication caused by bacterial contamination. The following experiments were devised

1 Action of sunlight and artificial visible light on the following aqueous solutions of pure amino acids was investigated —

Glycine, L-Alanine L-Valine L-Leucine L-Lysine L-Aspartic acid, L-Glutamic acid, L-Tyrosine L-Tryptophane and L-Histidine.

2. Action of solar light and artifical light (1000 watt bulb) on the aqueous solutions of following amino acids with sucrose employed as energy material was studied.—

Glycine, L-Alanine, L-Valine L-Leucine, L-Lysine, L-Aspartic acid L-Glutamic acid, L-Tyrosine, L-Tryptophane and L-Histidine

3 The effect of sunlight and artificial visible light on the following aqueous mixtures of amino acids with sucrose as additional source of energy was investigated —

Glycine + L-Alanine
Glycine + L-Valine
Glycine + L-Leucine
Glycine + L-Lysine
Glycine + L-Glutamic acid
Glycine + L-Tyrosine
Glycine + L-Tryptophane
L-Glutamic acid + L-Leucine

* This is an abstract of the thesis submitted to the Agra University for the degree of Doctor of Philosophy

4 The action of radiations available in sun and artificial visible light on aqueous solutions of the following amino acids in presence of colloidal MoO_3 and/or V_2O_5 was studied —

Glycine, L-Alanine, L-Valine L-Aspartic acid
L-Glutamic acid and L Tyrosine.

5 Action of ultraviolet light on tyrosine in presence or absence of colloidal Fe_3O_4 MoO_3 or V_2O_5 under present physico-chemical conditions and separately under nitrogen atmosphere was studied

Some of the significant results described in this thesis are as follows —

In all experiments completely sterilized solutions were studied to avoid any bacterial contamination

1 When sterilised aqueous solutions of glycine alanine, valine lysine aspartic acid, glutamic acid, tyrosine, tryptophane and histidine were exposed to sunlight and artificial light, it was noticed that two types of reactions took place simultaneously—photolysis of amino acids and formation of peptides. It was noticed that the photochemical action was more when exposure to sunlight was studied Further the rate of the action appeared, in general to increase when sterilised solutions were kept in quartz flasks No change in amino acid solutions was observed on keeping them in dark

2. The action of sunlight and artificial visible light (1000 watt bulb) was studied on sterilised aqueous solutions of glycine, alanine, valine leucine lysine, aspartic acid, glutamic acid tyrosine tryptophane and histidine in presence of sucrose employed as energy material. It was found that the photochemical action was of two types namely photolysis of amino acids and formation of peptides It was observed that presence of sucrose as energy material appeared to enhance the rate of both types of photochemical actions in all cases studied. It was noticed that the rate of photolysis appears to be effected more than the rate of photosynthesis of peptides. Artificial visible light was capable of synthesising only dipeptides in the mixture whereas sunlight could carry the synthesis in certain cases upto tripeptide on 600 hours exposure in quartz flask

3 The effect of sunlight and artificial visible light (1000 watt bulb) on aqueous solutions of alanine valine leucine, lysine, glutamic acid, tyrosine and tryptophane each mixed with glycine in presence of sucrose as energy material was investigated It was observed that the aqueous solutions of these amino acids mixed with glycine together with sucrose showed two types of simultaneous photochemical transformations—the pho[illegible] ino acids and the photosynthesis of peptides. It was observed that [illegible] nthesis [illegible] and of peptides was more when these mixtures of amino acids [illegible] dipeptides of dissimilar amino acids were formed more qu[illegible] tures than the dipeptides of the same amino acids. Photos

was favoured when the concentration of one amino a id was prominently high as compared to the other amino acid in the mixture The concentration of peptides of dissimilar amino acids which were stable in solution increased when the period of exposure was increased and the concentrat on of the peptides of the same amino acids appeared to decrease. These photochemical changes appear to be dependent upon the temperature range at wh ch expo ures were carried out. The solutions kept in the dark remained unchanged.

4. The action of sunlight and artificial visible light (1000 watt bulb) employed as physical agents for inducing chemical combinations in amino acids such as glycine, glutamic acid, valine, aspartic acid and tyrosine in aqueous solutions separately and in presence of colloidal MoO_3 and V O_5 as pos sible photosensitizers has been studied. Amino acids appea to undergo two types of reactions—photolysis and the formation of peptides. The oxide cata lyst appear to enhance the rate of both these types of ph tochemical reac tions in all cases studied The effect of these photocatalysts appears to be practically similar Only slight differences in the concentration of peptides and amino acids were observed in presence of different cat lysts. Photosynthesis of peptides in presence of oxide catalysts was more when sunlight was employed as the irradiation source and the solution was kept in transparent quartz flasks

5 The action of ultraviolet light on aqueous solution of tyrosine in presence of colloidal MoO_3, Fe_2O_3 or V_2O_5 in the present physico-chemical conditions, and, separately under nitrogen atmosphere was studied. For comparison, identical mixture of tyrosine v th MoO V O and/or Fe_2O was also irradiated with sunlight and artificial vis ble l ght. In presence of oxide photosensitizers—the two reactions viz photolysis and photosynthesis operate simultaneously It was noticed that in the case of exposure to ultraviolet, light, the rate of photolysis in the present physi-o-chemical conditions was slightly higher as compared to photosynthesis of peptides The rat of photosynthesis (ultraviolet exposure) appears to increase as ompared to sunlight employed for irradiation. When tyrosine with or without oxid catalyst was irradiated under nitrogen atmosphere, th rate of the formation of peptides appears to increase as compared to the rat f photolysis It was observed that at low temperature, the formation of p-ptides and photolysis of tyrosine were both sl ghtly hindered

The formation of peptides do take place when pure amino acids with or without sucrose as energy material or v th photosensitizers MoO_3, V_2O_5 and Fe_2O were irradiated with sunlight artificial light or ultraviolet light but the rate of formation was diff en with different physical agents and the vessel (quartz or pyrex) employed for irradiation.

Th control solutions kept in the dark were found unchanged in all cases studied

The formation of peptides in the above type of experiments appear to depend on formation of free radicals as intermediates.

These studies have a significant bearing on the chemical origin of life It is obvious that in any consideration of origin of life the formation of peptides from amino acids must be an important step towards the formation of proteins leading to the evolution of the cell. The experiments described here clearly help in visualising the possibility of formation of peptides from amino acids, even in the present physico-chemical conditions of the earth. One need not postulate a primitive atmosphere so far as the production of peptides from amino acids is concerned

STUDIES ON THE ELECTROPHORETIC PROPERTIES OF PROTEINS POLYSACCHARIDES AND THEIR DERIVATIVES*

INDU PRAKASH SAXENA
Chemical Laboratories D A V College Kanpur

The first statements regarding use of paper in electrophoresis are due to Paulo König These statements appear in his (Portuguese) publication[1] (1937) where it is mentioned that he employed ultra violet absorption for determining components separated electrophoretically Together with Klobusitsky[2] he separated (1939) by paper electrophoresis a yellow pigment from snake venom this was the earliest electrophoretic separation for protein Hence it is clear that paper electrophoresis is several years older than the modern form of paper chromatography whose beginning is marked by the 'Quantitative Analysis of Proteins A Partition Chromatography Method using Paper (1944) by Consden, Gordon and Martin.[3]

Important preliminary studies in this sphere then appeared in 1949 and as early as 1950 the value of paper electrophoresis was clearly demonstrated for the separation of mixtures of macromolecular substances. Later in the following years the publication of results obtained by a number of workers in this field attempted to explore this process nearly from all sides.

A rich and large deposit of experimental data is now available in the literature regarding the electrophoretic process as utilised in the investigation of properties of different types of organic and inorganic chemical compounds. But the reports published so far mention very little amount of ork regarding the role that the material of the paper plays in this process. It is not unreasonable to expect that different compounds would exhibit different electrophoretic behaviour on paper of different chemical composition.

It has been our intention in this investigation to remove this lacune A series of experiments therefore was carried out using a variety of papers to determine whether any difference in electrophoretic behaviour of the migrant could be observed which might be attributed to the nature of paper surface.

The present work, keeping in view the above mentioned intention has been classified as follows

(1) Comparative study of lectrophoretic behaviour of

(a) Sugars,

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(b) Amino acids,
(c) Organic acids, and
(d) Indicators

(2) Study of the effect of variation in the nature of paper used in the process for the above substances

(3) Study of effect and application of modified paper on some proteins and polysaccharides.

The following physico-chemical studies have been made for the different organic compounds and the paper utilised.

(1) *Ionic mobility*

Kunkel and Tiselius[4] are supposed to have put forward a complete theory advanced so far on electrophoresis.

In free solution the mobility U is

$$U = \frac{v}{Y} \qquad (1)$$

where v is the velocity and Y the field strength

Expressing this in the quantities usually measured on the paper

$$U = \frac{d\,l}{t\,V} \qquad (2)$$

where d is the distance V the voltage, l the length of the paper and t the time, or

$$U = \frac{d\,q\,K}{i\,t} \qquad (3)$$

where q is the cross-sectional area K the conductivity i the current and t the time.

The ionic mobilities (cm^2/V sec. $x\ 10^5$) of compounds on the different papers have been calculated on the basis of equation (2)

(2) *Isoelectric point*

It may be defined as a pH at which the molecule carries no charge To locate these points curves showing the behaviour of different compounds have been drawn between mobility and pH value

(3) *M value*

The relative rate of movement of the sugars is expressed as the M_G value where

$$M_G = \frac{\text{Distance of migration of substance}}{\text{True distance of migration of D-Glucose}}$$

(1) *Absorptive power*

About 20 ml of water were taken in a tube and weighed A paper strip (3×15 cm.) was cut and placed in that tube. This strip was allowed to stand vertically for six minutes. Some quantity of water was absorbed. The quantity absorbed in milligrams per minute by different types of paper indicated their absorptive power

(2) *Degree of acetylation*

It expresses the amount of acetic acid present per unit weight of paper and has been calculated only in case of acetylated papers.

The apparatus utilised in the laboratory for the present study was similar to that of Kunkel and Tiselius.[4] The paper strip was clamped between glass plates (41·0×16·5 cm.) supported on two electrode vessels (18×16 7× 6 cm) The two ends of the strip dipped into the buffer placed in the above mentioned vessels This apparatus was placed in series in both low and high voltage circuit according to the need

About 3% solution of different compounds besides a few was prepared in each case This solution was applied on paper strips by means of a capillary The strips were then dried and placed for the electrophoretic run in the above mentioned apparatus. After some time they were withdrawn, dried, stained and finally dried at a suitable temperature in oven.

The paper electrophoretic properties (distance of migration, ionic mobility isoelectric point and M_G value in case of sugars only) of substances mentioned previously[2-3] were studied on the following different types of paper

1 Untreated paper	—	(a)	Whatman No. 1
		(b)	Hand made.
2. Modified paper	—		Acetylated.
3 Impregnated	—	(a)	Alumina.
	—	(b)	Silicic acid.
4 Ion inclusion	—	(a)	Quinine
		(b)	Brucine.
5. Ion exchange	—		Resin treated.

It has been observed that the mobility of the same migrant was different on papers of different nature. This process, probably can no longer be regarded as analogus, in a limited sense, to Tiselius cell as suggested by previous workers who have hardly noted any difference in the electrophoretic behavior of different compounds on different types of paper

For instance, glucose which cannot be separated from xylose arabinose, galactose, sorbose and fructose at a certain pH value on whatman No. I

might be separated from the above sugars at almost the same pH value on hand made paper. Rhamnose and cellobiose which travel with equal mobility on whatman No. 1 at a certain pH range, might be separated from one another as well as from the remaining sugars migrating in the opposite direction on quinine treated paper at about the same pH range. The separation of glucose and fructose is possible on different papers at different pH values. But on alumina and silicic acid impregnated paper or quinine and resin treated paper these two travel comparatively with a wider difference in mobility at certain pH value.

It has been observed that almost on all types of paper studied glutamic acid travels nearly as fast as aspartic acid and as such their separation from one another does not appear to be easy. But on quinine treated paper the former may be observed to be stationary while the latter moving at fairly a high mobility. On alumina impregnated paper these two acids are observed to be stationary at the pH range 2·6-7·4. This fact which is not observed on other types of paper can be utilised in their separation from other amino acids.

Quinine treated papers appear to be comparatively a better material for separation of both the types of ions migrating towards anode and cathode. In addition to this compact spots of different substances are revealed on this paper.

A comparative study of protein of egg white of hen, duck and guinea fowl was made on quinine treated and whatman No. 1 paper. It was observed that protein obtained from egg white of duck was composed of two parts, one migrating towards anode while the other towards cathode at pH 6·1 and 7·5. This fact was not observed on whatman No. 1. Similarly at pH 7·5 protein obtained from egg white of guinea fowl could be separated on quinine treated paper more distinctly than on whatman No. 1. The egg white protein of hen migrated into two parts one heading towards anode while the other towards cathode on the above treated paper. But on whatman No. 1 it migrates as one part in one direction.

The above observations show that better separations of different types of compounds are possible by ordinarily known paper electrophoretic methods. This process can be developed as a newer analytical tool for purification and identification of different compounds.

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STUDIES ON THE PATHOLOGY OF AVIAN LEUCOSIS COPLEX

R P AWADHIYA
Department of Pathology & Bacteriology
U P College of Veterinary Science and Animal Husbandry Mathura

Investigations were undertaken to find out the occurrence of avian leucosis complex and also to study the pathology of the various forms of the condition encountered in the Poultry Farm at U P College of Veterinary Science and Animal Husbandry Mathura Attempts were also made to a limited extent to study the susceptibility of Desi fowls to avian leucosis complex by experimental studies.

The tissues were collected from liver spleen kidneys lung heart intestine pancreas, ovary testes sciatic nerves brachial plexus and bone marrow of radius from the birds which were submitted for the routine post mortem examinations conducted in the Pathology and Bacteriology Department and showed gross lesions of avian leucosis complex. The report on the incidence of avian leucosis complex was prepared for a period of five years from January 1955 to December 1959 from the post-mortem records conducted in the Department of Pathology and Bacteriology The chickens of indigenous (Desi) breed which were raised by hatching eggs of Desi fowls were utilized for the experimental transmission trials.

The lesions in 47 natural cases of avian leucosis complex were classified on the basis of the histopathological examination as Visceral lymphomatosis (37) Myeloid leucosis (5) Erythroleucosis (1) Erythro-myeloid leucosis (1) Ocular lymphomatosis (1) and Osteopetrosis (2)

The lesions of visceral lymphomatosis were categorised as diffuse and nodular types The lesions of diffuse type were characterised by great enlargement of the various organs particularly spleen and liver with or without the presence of grey foci Histopathological examination of the lesions in such cases revealed extravascular massive accumulation of large mononuclear cells having large nucleus with little chromatin material mainly located at the nuclear membrane. The lesions of nodular type of visceral lymphomatosis were characterised by the presence of few large tumour nodules without much enlargement of the affected organs. Histopathology of such lesions was characterised by extravascular accumulation mainly of the lymphocytes possessing condensed chromatin material in the nucleus and relatively large cytoplasm

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enlargement of the cortical and medullary cells of the stolon tips The transition from stolon to tuber is very gradual. What factors determine tuberization have not been studied so far But light intensity temperature and amount of water available seem to play an important role in this connection

Like other monocotyledons *Cyperus esculentus* has an adventitious root system Roots show a polyarch structure with a sclerenchymatous hypodermis. In their ontogeny the root primordia develop in early stages of axis formation. They develop from a group of small initial cells which appear to be formed by irregular divisions in the ground parenchyma of the vertical axis. The root apex shows three sets of initial cells One set forms the root stele, the second protoderm-cortex and the third calyptrogen The structural configuration at the root apex thus falls under the Type two of Esau (1953)

The leaves of *Cyperus esculentus* are differentiated into a small sheathing leaf base and a long linear blade with a pointed tip The lamina is grooved and presents a 'V' shaped structure. The silica cells are characteristic in having conical silica depositions that have crescentic outgrowths at the bases. Large longitudinal air canals run throughout the leaves and are interrupted at irregular intervals by horizontal diaphragms

The leaves originate from a group of initial cells of the superficial cells of tunica To begin with every cell of the leaf primordium is meristematic but later on the basal most cells mature first and the tip remains meristematic

The inflorescence axis or the culm is distinguished by its considerable length and triangular outline It is exceptionally straight and does not show any branching It presents a 'Y' shaped structure in cross section Like leaves in it also run the longitudinally running collateral vascular bundles.

Diaphragms are specially characteristic of leaves but occur in the culm also Th structure of diaphragms varies as they may be one to several layers thick, th cell may vary from polygonal to stellate. They are always supported by a cross-bundle that connects the two longitudinally running bundles of the leaf or culm. Diaphragms arise by a divis on of the ordinary pa ench matous mother cells.

Inflorescence is in the form of an umbellate, peduncled spike composed of a large number of leaf bracts prophylls peduncles and spikelets. There i one proph ll in each spikelet There are two empty scales at the base of each spik let. The lower of these empty scales is a reduced leafy bract from the axil of which the ray or the peduncle arises The upper empty tubular scale sheath has been regarded as th prophyll.

Spik lets are pindle shaped s ructures and bear a number of g\ in the axils of which ar present the flowers The fl wers are of two t)

hermaphrodite and pistillate. Either all the flowers are hermaphrodite in a spikelet or when mixed the pistillate at the base and hermaphrodite near the apex of the spikelet. They are spirally arranged

Each hermaphrodite flower has 5 to 6 perianth lobes, three stamens and a tricarpellary gynoecium. In pistillate flowers the stamens are replaced by staminodes.

Ontogenetic studies reveal that the cells of the apex of the spikelet in *Cyperus cuculatus* are arranged in a two layered tunica covering a central corpus. The outer and inner tunica layers have differently been termed as dermatogen and hypodermis. Periclinal divisions in the hypodermal cells and subsequent divisions in the dermatogen give rise to the glumes and carpels. Periclinal divisions in the hypodermis form the stamens. Terminology of Barnard (1957) has been followed

In the flowers the perianth members are regarded as true perianth which do not have any vascular supply The stamens are three arranged in the outer whorl.

The structure of carpel is normal and is composed of a tricarpellary ovary with a single basal ovule a dilated style and a trifid stigma The single basal ovule because of its central position and compound vascular supply is believed to have been derived from a free-central ancestry and as such offers support to Blaser's view (1941)

The present observations on the embryology reveal that in the microsporogenesis the spore mother cell divides reductionally to form four nuclei. Out of these four microspore nuclei the three degenerate completely while the fourth forms the functional microspore nucleus. There is no septum formation for separating these degenerating and functional microspores. The pollen grains are tenui-exinous.

A single hypodermal archesporial cell differentiates in the early ontogeny of the ovule formation. It divides into a parietal cell and a megaspore mother cell The development of the embryo sac is *Polygonum* type. The number of antipodals is three and they are linearly arranged.

I have the pleasant duty to express my gratitude to my respected teacher Prof V Puri, D Sc., F.N I for his valuable guidance, generous help encouragement and unfailing kind interest in the problem throughout, which has made this work possible Thanks are also due to the authorities of C. S. I. R. for the grant of a Junior Fellowship during the tenure of which this work was done.



STUDIES ON THE ACCESSORY REPRODUCTIVE GLANDS OF SOME HELMINTH PARASITES OF DOMESTICATED LIVESTOCK*

G SUBRAMANIAN
Post-Graduate College of Animal Sciences
Indian Veterinary Research Institute Izatnagar U P

This thesis embodies the results of the investigation into the histochemical nature and functional morphology of the accessory reproductive glands of five species of helminths. The nature and mode of formation of the protective envelopes around the eggs in these parasites has been studied by histochemical methods

The Mehlis gland cells and the vitelline glands are associated with the egg producing genitalia in most of the trematodes and cestodes. Mehlis or "shell" gland was originally believed to secrete the egg-shell or capsule in the trematode egg The vitellaria in the trematodes and pseudophyllidean cestodes are now known to provide the bulk of the egg-shell precursors (Smyth & Clegg 1959) The completed egg-shell in some of the trematodes and pseudophyllidean cestodes has been shown to be a quinone-tanned scleroprotein (Stephenson, 1947 Smyth 1956 Smyth & Clegg, 1959)

Although the Mehlis gland cells do not provide any material for egg-shell formation, its location around the ootype is suggestive of some physico-chemical relationship with the process of egg-shell formation. Rao (1959a, 1959b, 1960b) has recently suggested that the Mehlis gland secretions are responsible for the extrusion of the shell globule matter from the vitelline cells

In the present work the histology and histochemistry of the female genitalia in the trematode *Gastrothylax crumenifer* (Creplin 1847) the cyclophyllidean cestodes *Moniezia expansa* Rudolphi, 1810 *Avitellina* Gough, 1911 and *Stilesia globipunctata* Rivolta, 1874 and the nematode *Ascaridia galli* Schrank, 1788 have been studied. In all, thirty histochemical and staining reactions have been applied and the observations are summarised in tables 1 to 6 and analysed in detail in the text

The Mehlis gland cells in *G crumenifer* and *M expansa* show close resemblance and they give positive tests for carbohydrate-protein complexes unsaturated fatty acids, and ribonucleic acid or ribonucleoprotein.

In *G crumenifer* the following three important physico-chemical processes occur in the ootype region :

*Abstract of the thesis submitted to Agra University in fulfilment of the requirement of the degree of Doctor of Philosophy

1 There is extrusion of the egg-shell precursors and their physico-chemical reorganisation

2 Simultaneous release of the vitelline granules from the shell globules prior to the extrusion of the latter and

3 Chemical changes occur in the contents of the vitelline cells when they are included in a fully formed egg

It has been shown that the Mehlis gland secretions are responsible for bringing about the above mentioned changes. Furthermore it has been demonstrated that a part of the Mehlis gland secretions is also incorporated into the egg-shell

There is evidence that there is lysis of the shell globules inside the vitelline cells prior to their extrusion into the ootype. After their release, the shell globule matter agglutinates into a refringent mass which forms the egg-shell. The globules are fibrin or fibrinoid type of protein spheres.

Soon after the egg-shell is formed the shell material loses its basophilia indicating that the protein end groups are blocked. The surficial layers of the egg-shell at the same time become positive for unsaturated lipids, although this component is not evident in the shell globules There is no evidence of 'keratinisation (disulphide (SS) linkages) in the protein moiety of the egg-shell

The phospholipid-like secretions of the Mehlis gland cells are absorbed into the vitelline cells when these reach the ootype. While within the vitelline cells, these secretions cause extrusion of the shell globule matter through a lytic type of reaction Rao (1959b) did not indicate the location of the lysolecithinase type of enzyme. As it is improbable that the enzyme and its substrate (Mehlis' gland secretions) are secreted by the same glandular cells it is suggested that the lysolecithinase is contained within the vitelline cells The interaction between the lecithin type of secretions of the Mehlis gland cells and lysolecithinase produces lysolecithin The latter causes lysis of the hell globules. During lysolecithin formation an unsaturated fatty acid component is also liberated (Zeller 1951) This unsaturated fatty acid is presumably incorporated into the shell globule matter and this mass permeates into the lumen of the ootype due probably to osmotic imbalance

With the extrusion f the shell globul matter the lysolecithin in the vitelline cells interact with the vitelline granules and the resulting product is strongly PAS PAAS nile blue and Sudan Black B positive thus showing it to be an unsaturated lipid and serving as the reserve food material during embryonation

In *M. exposita* the vitelline cells do not produce any egg-shell precursors and hence substrates similar to those of *G. crumenifer* are n t present. Consequently the Mehlis gland secretions have no apparent function in the form

tion of the protective envelopes of the egg-cells. From these observations, it is concluded that the Mehlis gland cells in *M. expansa* are vestigial structures.

The uterus in *M. expansa* and the uterus and paruterine organ in *Avitellina* sp. and *S. globipunctata* provide the protective envelope, the chorion to the egg-cells in addition to giving nourishment and protection during embryogeny. The chorion originates from cellular elements and secretions of the uterus in *M. expansa* and in the paruterine organs in *Avitellina* sp. and *S. globipunctata*. There is reorientation, aggregation and stabilisation of the fibrillar elements and secretions. The chorion is composed of collagen and fibrin in *M. expansa* and collagen and elastin in *S. globipunctata* and *Avitellina* sp. The protein moiety of the chorion is stabilised by disulphide (SS) linkages ("keratinisation").

In addition to the chorion the mature egg of these cyclophyllidean cestodes is provided with a second protective envelope, the embryophore, which originates endogenously from a few micromeres of the developing embryo. The embryophore is composed of elastin type of scleroprotein which is stabilised by disulphide (SS) linkages ("keratinisation").

Gough (1911) reported the presence of nutritive cells in the ovary of *Avitellina centripunctata* but in the present investigation no such cells could be identified by histochemical methods in *Avitellina* sp. and *S. globipunctata*. The present study however confirms and extends the observations of Gough (1911) regarding the presence of nutritive elements in the uterus.

In *A. galli* the reserve material is present in mature oocytes which following fertilisation, undergoes physico-chemical reorganisation to form the egg-shell. The reserve materials give positive tests for protein-carbohydrate complexes, glycogen, ribonucleic acid and lipids. The fully formed egg-shell is composed of a reticular framework of elastin type of scleroproteins and whose intermeshes are filled with acetylated chitin. This acetylated chitin is negative to PAS reaction but is positive when first formed due to its being non-acetylated in the beginning. The surficial layers of the egg-shell show the presence of protein-carbohydrate complexes and lipids and the enclosed reticular matrix making up the thickness has chitin components. The evidence for quinone tanning of the surficial layers is not conclusive.

THE PHENOMENON OF SUPERPARASITISM IN *TRICHOGRAMMA EVANESCENS MINUTUM* RILEY AND *BRACON GELECHIAE* ASHMEAD*

M. J. Chacko†
Indian Agricultural Research Institute New Delhi—12

A good deal of controversy exists about the usefulness of *Trichogramma evanescens minutum* in the biological control of insect pests. The objections raised against this parasite are its limited range of operation, its random search for hosts and its non-specificity. The author's[1] own earlier observations have led him to believe that possibly superparasitism which occurs when *Trichogramma* is mass-multiplied and which affects the fecundity and longevity of this parasite is another important factor that limits the efficacy of *Trichogramma* in the biological control of insect pests. Very little information is available on the fecundity and longevity of *Trichogramma* that emerge from superparasitised host eggs. The present investigation was, therefore, undertaken to study the effect of superparasitism on these aspects. Superparasitism also occurs when *Bracon gelechiae* is mass-multiplied in the laboratory. Studies on the effect of superparasitism in this parasite have also been made.

For experiments on *Trichogramma* two different strains, *viz.* IARI strain (from the culture maintained at the Parasite Laboratory of the Indian Agricultural Research Institute) and Ajmer strain (obtained from eggs of *Chilo zonellus* Swinhoe collected on jowar at Ajmer) and the crosses between them (IARI male × Ajmer female, and Ajmer male × IARI female) were used. The fecundity and longevity of the following have been studied in detail —

1. *Trichogramma* that develops singly in an egg (of *Corcyra cephalonica* Stainton) without any superparasitism.
2. Apparently normal female *Trichogramma* that develops along with an apparently normal male in the same host egg.
3. Apparently normal female that develops along with another apparently normal female in the same egg.
4. Defective female that develops along with a defective male in the same egg.
5. Defective female that develops along with another defective female in the same egg.

* Summary of thesis accepted for the Ph. D. degree of Agra University

† Present address: Commonwealth Institute of Biological Control, Indian Station, Hebbal, P. O. Bangalore—6.

1 Narayanan, E. S. & Chacko, M. J.—Superparasitism in *Trichogramma evanescens minutum* Riley (Hymenoptera: Trichogrammatidae), an egg parasite of sugarcane and maize borers in India. I. Effect of superparasitism. *Proc. Ind. Acad. Sci.*, XLV, No. 3, Sec. B, 1957, 122-128.

These studies were made for the two different strains as well as for the crosses between them and for three successive generations (except in 4 and 5 where no defective second generation was procured) Eight replications were made. Separate cultures of the two different strains and the crosses between them were maintained Each culture was started from a single pair and only the progeny of this have been used for experimental purposes.

A separate culture of *Bracon gelechiae* was started from a single pair from the culture maintained at the Indian Agricultural Research Institute for studies on this parasite The development of 4 or 5 specimens of *B gelechiae* on a fully developed larva of *Corcyra* is normal In order to find out the effect of superparasitism 4 6, 8 10 12 and 14 eggs were allowed to start development on a single host caterpillar and the fecundity and longevity of three successive generations were studied Eight replications were made. Studies were also carried out to find the effect of superparasitism on development, emergence sex-ratio developmental period and size

All the experiments were conducted at a temperature of 25°C and at a relative humidity of 75 %

Observations and Conclusions

Trichogramma evanescens minutum. It is evident from the data obtained during the course of the present investigation that superparasitism has a very adverse effect on the fecundity and longevity of this parasite When *Trichogramma* develops singly in an egg of *Corcyra* it gets sufficient nourishment which enables it to emerge as a normal adult Its fecundity and longevity are not impaired There is no marked difference in the fecundity or longevity from generation to generation if the development continues to be normal However a reduction in fecundity and longevity is observed when two apparently normal parasites are produced from one host egg When two parasites develop together in a host egg the amount of food available within the host is shared by the developing parasites with the result that neither of them gets sufficient nourishment to become a normal adult. This undoubtedly affects the longevity and fecundity which are reduced And the reduction is maintained in the succeeding generations (if two parasites develop together) An interesting observation is that when superparasitism occurs and when the two parasites that develop together belong to the opposite sexes then the fecundity is less than that of any of the two females that develop together It is possible that the males have a quicker rate of food intake than the females Therefore a female that develops along with a male gets lesser food than either of the females that develop together because in the latter case the two developing females share the food more or less equally This may account for the higher fecundity of the females that develop together Whenever superparasitism occurs with the production of defectively formed adults there is a distinct reduction in the fecundity and longevity Probably

more than two parasites start sharing the food initially with the result that the two survivors do not get even as much food as the two that start sharing the food initially It has also been observed that the fecundity and longevity of the Ajmer strain are considerably less than those of the IARI strain. Probably this is owing to the fact that the Ajmer strain was originally obtained from the eggs of *Chilo zonellus* and have not become quite adapted to the eggs of *Corcyra* whereas the IARI strain has been adapted for years to development in *Corcyra* eggs. The fecundity of the cross IARI male X Ajmer female is higher than that of Ajmer male X IARI female The combined fecundity of two parasites that develop in a host large enough for one is found to be less than that of one developing in a host.

It is clear from the above that the ill effects of superparasitism have a direct bearing on the efficacy of *Trichogramma* as a biological control agent. In the laboratory breeding of this parasite superparasitism inevitably occurs and when such parasites are used for the control of insect pests we can hardly expect any success Therefore, for the laboratory rearing of this parasite it is necessary to develop a method whereby the incidence of superparasitism can be lessened, if not avoided altogether

Bracon gelechiae Superparasitism has an adverse effect on the fecundity and longevity of this parasite also. As the number of parasite grubs that develop on an individual *Corcyra* larva increases, the amount of food available is shared by the developing parasites and thus the grubs are deprived of their normal food requirements. When 6 or more grubs develop on a host the fecundity and longevity of the adults are significantly less than those of 4 that develop on a host. When 6 to 10 parasites develop on a host, there is no significant difference between their fecundity or between their longevity however the reduction in fecundity and longevity when 12 to 14 develop on a host is significant. It has been observed that the maximum number of parasites that completes larval development and emerges successfully is never more than eight. Since several of the grubs do not complete development superparasitism results in a wastage of progeny When there is heavy superparasitism some grubs are pushed aside resulting in their death, others that apparently complete larval development do not form a cocoon some that succeed in pupating do not emerge while others emerge as runts As a result of superparasitism a preponderance of males has been observed the percentage of males varies from 35 48 when 4 parasites develop on a host, to 78·72 when 12 develop on a host. Similarly the developmental period increases from 9 63 days when 4 develop on a host to 13 63 days when 12 develop on a host. In *B gelechiae* dwarf forms are produced only very rarely when superparasitism occurs.

These effects of superparasitism on fecundity longevity development and emergence sex ratio and developmental period are factors that play an important role in the successful propagation of this parasite in the labo-

ratory In the mass production of this parasite superparasitism should be avoided as far as possible, since superparasitism results not only in a wastage of progeny but also in the production of defective forms with their reproductive capacity adversely impaired.

SOLID STATE SPECTRA OF URANYL SALTS*

DEVAKI NANDAN PANDE†

Physics Department D S B Govt Coll ge Naini Tal

The uranyl ion (UO_2^{++}) is mainly responsible for the fluorescence and absorption of uranyl salts. A given salt however sometimes shows changes in its spectrum and some new bands besides the usual ones occur depending upon th conditions of preparation of the various samples. These are obviously due to some impur ties in the bulk material The nature of these impurities has not been studied in the past. In solutions uranyl salts are known to undergo hydrolysis and complex formation with anions with corresponding changes in their spectra. It is therefore, likely that some of these new species are stable in the solid state also and thus may act as impurities.

The fluorescence and absorption spectra of the various samples of uranyl chloride, prepared under various conditions, have been investigated at —185°C and also with rising temperature. Evidence has been found for three distinct stable species in the solid state. The species (S_1, S_2 and S_3) have been isolated and the conditions under which they are formed are specified. In sample S_1 obtained from acidic solution of the original salt, UO_2^{++} ion probably exists as such and gives the usual series of bands B C, E etc. The first hydrolysed product S_2, obtained as a precipitate from concentrated aqueous solutions at about 80°C gives new sets of bands D etc. resembling the γ* bands of alkaline solutions reported by earlier workers The highe hydrolysed product S was obtained after evaporat on of aqueous solution of the original salt at 120°C. It gives new B C' series of bands resembling the δ bands of solutions. These products are generally present as impurities in the various samples. Relative to the (0, 0) band of S_1 those of S_2 and S_3 are shifted to the red by 200 and 1315 cm^{-1} respectively The vibrational intervals of S_2 and S are smaller by 26 and 70 cm^{-1} respectively in comparison to that of S_1 In absorption the intervals for the hydrolysed products are again smaller than for the normal salt. The temperature variation of the fluorescence intensity of these hydrolysed products in solid state resembles closely the behaviour in solutions and intensity falls rapidly with rise in temperature.

The acetate also gives hydrolysed products like chloride on crystallising from aqueous solutions or even in contact with atmospheric moisture. It also forms anionic complexes as in double acetates in presence of excess of acetic acid. It has been proved that the energy hifts of the electronic part on successive hydrolysis in case of chlorid and acetate are not only qualitatively

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† Present address Lecturer in Physics, Jodhpur University Jodhpur

but also quantitatively similar for I and II hydrolysed species with respect to the normal salts. The electronic energy on anionic complexing changes to the higher values relative to that of the normal salt in agreement with earlier observations in connection with solutions as by Pant and Khandelwal and in double acetates. The similarities of the hydrolytic and anionic complexes in solutions and solids are further corroborated by the study of other properties such as temperature variation of fluorescence intensity and qualitative changes in the relative intensities in the absorption bands. The various forms of acetate are sometimes present together in different proportions and act as impurities for each other. In some cases it is observed that the impurity bands are shifted relative to their positions in pure samples which points out to the formation of mixed crystals where the impurities are either substitutional or are present as interstitial in the lattice. Interesting characteristics of the impurities have been observed and studied in some detail.

Uranyl sulphate is also known to show spectral changes depending upon the methods of crystallisation dehydration etc. In earlier literature there was a confusion in the observations of various workers. An exhaustive study of the various samples of this salt prepared under different conditions has cleared up the situation in many respects and the earlier observations have been correlated to a great extent. At least eight distinctly different forms of uranyl sulphate have been recognized by their spectral and other properties. The various phase transformations are found mainly either due to differences in the amount of water of crystallisation or due to different possible crystal structures for the same chemical composition and in one case due to the anionic complexing. It has been proved from monochromatic excitation intensity changes with temperature etc. that the α and β series (A and B series) in the samples of uranyl sulphate trihydrate belong to a single hydrate in contrast to the suggestions by some earlier workers who attributed these bands to two different hydrates present in the same sample. One form of the salt obtained on dehydration with H_2SO_4 gives the antisymmetric frequency (ν_3) of uranyl ion in as many quanta as the symmetric one. This observation is extraordinary as all other uranyl salts are known to give only a single quantum of ν_3. The spectral changes during various phase transformations have been described.

It is now becoming clear that the uranyl ion is stabilised by an equatorial ligation which may be due to water of hydration or other ions of the crystal. Due to this ligation not only is there a shift and change in the spectrum from salt to salt but also new vibrations which may be called internal lattice modes or ligand bond vibrations can arise. We have recognised the possibility of such ligand vibrations in the spectra of solids. Vibrational frequencies about 90 and 300 cm^{-1} are attributed to different lattice modes and evidence for this suggestions is gathered. The systematic changes in the electronic energy involved in the transition corresponding to fluorescence are accompanied by similar changes in the vibrational frequency of the uranyl

ion in the ground state. The relation ν ν_1 is found almost parabolic for the two series of salts, namely chlorides and nitrates. There has been no difficulty in the identification of the symmetric frequency (ν_1) of the uranyl group in fluorescence as it occurs strongly in several quanta. The regions of the spectra corresponding to the other frequencies (ν_2 and ν_3) show multiplet structures in several cases and it is ordinarily difficult to indentify these latter frequencies unambiguously. The behaviours in the higher groups show that the series belonging to ν_3 frequency gains in relative intensity over the other bands in the respective regions. The ν_3 frequency was thus indentified and an empirical relation between ν_3 and ν_1 has been deduced which represents the experimental results within 1.5% in most of the cases. A similar relation is given for ν_2 and ν_1 frequencies. The other bands occurring in the regions of ν_2 and ν_3 frequencies have been attributed to the uranyl-ligand bond vibrations. In few cases the frequencies calculated from semi-empirical assumptions are in accord to the experimentally observed values. The causes for the occurrence of the ν_1, ν_2 and ν frequencies together have been enumerated. Two series of bands at about 90 and 300 cm^{-1} have been interpreted as out-of plane bending and symmetric stretching modes respectively of the uranyl-ligand bonds. They occur with relatively greater intensity in compounds where the ligands are probably covalently bound with the uranyl ion, e. g. in double nitrates and double acetates. In the cases where water is directly coordinated with U-atom these vibrations have been recognised by D_2O substitution.

In absorption the four series of electronic transitions in the region 5000 to 3400 A° have been identified in many of the spectra. It has been found that like in solutions the magnetic series gains in relative intensity over the diffuse series on anionic complexing and becomes less intense on hydrolysis. The magnetic diffuse and ultraviolet series show multiplet structures invariably and in few cases the fluorescence series also behaves similarly. The schemes suggested by some earlier workers are untenable for the interpretation of the uranyl absorption. The fluorescence series show the occurrence of the antisymmetric vibration ν' in as many quanta as ν. These uranyl vibrations follow a similar relation as the corresponding ground state vibrations. Some of the contradictions in the earlier literature about the excited state vibrations are thus refuted.

D_2O isotopic shifts in the spectra of a few hydrated crystals of uranyl salts have been investigated with a view to understanding the role of the water of crystallisation in these salts. In agreement with the earlier observations of other workers the entire spectra are shifted by 8 to 20 cm^{-1} to the violet. However in case of $UO_2Cl_2 \cdot 3H_2O$ there is observed no shift in contrast to other cases. It is suggested that the violet shift is due to change in the zero-point energies in the ground and excited states of the uranyl complexes in which water is coordinated with the uranyl group more or less strongly. Evidence has been found for the occurrence of uranyl-ligand vibrations involving water molecules as ligands. The ligand vibrations are found to show

larger shifts which are in accord with the theoretically expected values The conclusions are supported by semi-quantitative derivations of the ligand bond vibrational frequencies from Badger's rule and bond strength relations suggested by earlier workers. It is suggested that the D_2O isotopic shifts can be more fruitfully studied at still lower temperatures and higher dispersions.

Polarization studies in the fluorescence and absorption spectra of a few single crystals of uranyl salts have been undertaken to get informations about the symmetries of the vibronic states of the uranyl group in the ground and various excited states Except the cubic crystals of sodium uranyl acetate, which show circular polarization in a single series of bands (Symmetric vibrations) all the lower symmetry crystals show strong dichroism in some or the other settings of the individual crystals. The dichroism is due to strong polarizations of the magnetic and diffuse series of absorption bands which can be thus easily distinguished. The behaviour of the diffuse series is somewhat different than that of the magnetic series The fluorescence and ultraviolet series are also more or less polarized. The most interesting point is that the ν_1 and ν_3 vibrations of the uranyl group in the first excited state are polarized perpendicular to each other while in the ground state only ν_3 is strongly polarized This shows that the UO_2 group is in all probability of $D_{\infty h}$ symmetry in the ground state and its geometry changes to C_{2v} on excitation. The sodium uranyl acetate crystal show two types of circular polarizations namely left and right handed The two polarizations are due to the enantiomorphism in this class of crystals. It is suggested that in this particular case the UO_2 group remains linear symmetrical in the excited state as well and the circular polarization is due to electronic transition most probably from Δ_g upper state to $1\Sigma_g^+$ ground state. The possible electronic configurations of the excited uranyl ion are discussed briefly

STUDIES IN POLAROGRAPHY*

P. K. Karmalkar

Holkar College, Indore

Chapter I *The determination of Stability Constants of Inorganic Complex Ions in Aqueous Solutions*

In this chapter a general survey of the historical background of finding out of stability constants is taken. The following points are further discussed.

(i) It is necessary to abandon the aim of obtaining constants at infinite dilution when studying complex equilibria where several species exist simultaneously.

(ii) It is necessary to employ a constant ionic medium for the study of complicated equilibria in order to eliminate activity coefficients as variables. Any limitations that the ionic medium method may have are certainly outweighed by this advantage.

(iii) In order to establish definitely the presence or absence of polynuclear complexes it is necessary to study the equilibria over a wide range of ligand concentration. The central ion concentration should also be varied over a wide range.

(iv) Since one must reproduce the experimental data with the minimum number of constants and assume that these indicate the correct ionic species, it is essential to have highly accurate data to avoid the introduction of ficticious complexes.

Chapter II *New methods for the Estimation of the Stepwise Stability Constants of Complexes*

Today it has become a practice of applying statistical methods for computing various chemical constants. Kivalo and Rastas have proposed a method for calculating stability constants of complexes based on 'least-square method'. Here I have proposed two new statistical methods based on the theory of 'orthogonal polynomials' for computing stability constants of complexes. The methods are suitable for the determination of stability constants of moderately weak mono-nuclear complexes when the ligand concentration is large compared to the central ion concentration and is varied at constant intervals.

The limitations of 'least square' method as proposed by Kivalo and Rastas are discussed and the superiority of the proposed method—that there is no loss of labour in this method as in least square method—is shown. It is

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*This is an abstract of the thesis submitted for the degree of Doctor of Philosophy of Agra University

because the orthogonal polynomial equations involve a transformation of such a nature that as new constants are added the old constants remain the same.

This point of superiority is brought out by applying the Kivalo and Rastas method and the proposed new methods to the data of copper-acetate system ($\mu = 0.5$)

The standard errors of coefficients are found whereby the number of complexes existing in aqueous solution could be judged.

Chapter III *Polarography of complex metal ions—Their Composition and Stability (or Instability) Constants*

In this chapter the classical polarographic method as applied to a system when a comparatively strong complex exists over a wide range of ligand concentrations is described. The method, as modified to suit cases when weak acids (as acetic) or amino acids are used is discussed.

When several weak complexes are formed in a stepwise fashion the above method fails For such cases two methods are available *viz.*, (1) De Ford and Hume's and (2) Ringbom and Eriksson's. These are described in details.

Chapter IV

In this chapter the main polarographic set up and the auxiliaries required for the same are discussed fully to give a clear idea about the general polarographic experimental set-up.

Chapter V *Polarographic Studies in the Mono-Carboxylate Complexes of Cadmium.*

Ferrel et al investigated potentiometrically the electrolytic dissociation of copper and cadmium carboxylic acid salts. They studied the Anisophillic and Katiophetic properties of cations and anions. However their aim was not to find the formation constants of the cadmium-mono-carboxylate complex ions Aditya and Prasad in their studies on the behaviour of Bi univalent salts in aqueous solutions studied the cadmium acetate system. Hershensen et al studied the formate complexes of cadmium copper lead, thallium and zinc polarographically

Recently Klemenčić and Fillipović studied polarographically lead monocarboxylate complexes and their stability complexes In the present work similar study has been undertaken for cadmium mono-carboxylate complexes with formate acetate propionate and butyrate ions with a view to determine their stability constant as also to see the influence of increasing chain length in homologous series on the number of ligands—attached and the stability of complexes.

It has been found that cadmium forms three stepwise complexes with all the anions studied—formate, acetate propionate and butyrate in the concentration range of (0·0-1·0) M although in case of butyrate, presence of higher complex ions is suggested and this is expected as will be clear from the discussion.

The overall successive formation constants are as follows:—

Formate system	$\beta_1=4$	$\beta_2=15$	$\beta_3=14$
Acetate system	$\beta_1=18$	$\beta_2=30$	$\beta_3=167$
Propionate system	$\beta_1=6$	$\beta_2=88$	$\beta_3=176$
Butyrate system :	$\beta_1=5$	$\beta_2=50$	$\beta_3=226$

But for the discrepancy in β_1 for acetate, it has been found that, n general the values of formation constants increase with increase in chain length in homologous mono-carboxylate acid system.

In general the stability of complexes stand as formate < acetate < propionate > butyrate

This is in order as the dissociation constant of these acids which is also as : formate < acetate < butyrate < propionate

Moreover the relation between the stability of complexes and the dissociation constants of the acid is obscure and there could not be traced any quantitative relationships between the two

Chapter VI *Polarographic and Spectrophotometric Studies of the acetate complexes of copper*

Fronaeus first studied the acetate complexes of copper by cation-exchange resins. He made measurements with cupri acetate solution at an ionic strength of 1M (with $NaClO_4$) and found the presence of complexes $Cu(oAc)^+$ $Cu(oAc)_2$ and $Cu(oAc)^-_3$ with the respective formation constants of

$\beta_1 = 45 \pm 2c^{-1}$ $\beta_2 = 440 \pm 60$ c^-

$\beta_3 = 1000 \pm 300c^{-3}$

Lloyd et al from solubility data, studied the dissociation constants of cupric salts of some monocarboxylic acids They report the dissociation constant (reciprocal of formation constant corrected for ionic strength as $k = 5 \cdot 7 \times 10^{-3}$

Sircar and Aditya studied the copper-acetate system, potentiometrically and report the presence of first two complex ions $Cu(oAc)^+$ and $Cu(oAc)_2$.

Recently Y Doucet and R. Cognlac, by conductivity method, studied the above system and they report a value of $3{\cdot}0 \times 10^{-3}$ for k_1

N Doucet et al from optical density measurements report that copper (II)-acetate solutions of concentration 0·1M behaves as 1—1 electrolyte as Cu(oAc) +(oAc)$^-$ with no free Cu^{++} ions existing in these solutions.

In all the above works referred to methods other than polarographic were used and the conditions of experiments used by different workers were also different.

In the present investigation copper acetate complexes were studied polarographically and spectrophotometrically

In polarographic method the data was analysed by De Ford and Hume's method both for establishing the number of complexes and their formation constants Three series of aqueous solutions containing copper 5 × 10^{-4}M and different concentrations of acetate ion kept at constant ionic strengths of 0·15 0 5 and 1 5 respectively by means of KNO_3 were studied.

In lower ligand concentrations series, only the first two of the above complexes were detected. The successive formation constants β_1 and β_2 having values 107 and 340 for μ=0·15 and 82 and 385 at μ=0·5 In high ligand concentration the following complexes were detected Cu(oAc)$^+$ Cu(oAc)$_2$, Cu(oAc)$_3^{-1}$ Cu(oAc)$_4^{-2}$ with respective overall formation constants of 82, 300 91 and 46 respectively The formation constant of the ion Cu(oAc) $_5$ is small—too small to be evaluated with any degree of confidence by this method.

In the spectrophotometric method, Yatsimirskii's method is used Two Series of copper acetate system—one at higher ionic strength (μ=3·0M) and another at lower ionic strength (μ=0·15 M) were studied. In the lower range of acetate ion concentration (0·1M) the presence of only two complexes Cu(oAc)$^+$ and Cu(oAc)$_2$ is inferred with values for successive overall constants β_1=100 and β_2=350 In the higher concentration range (μ=3M) series presence of as many as first five complexes Cu(oAc)$^+$ Cu(oAc)$_2$, Cu(oAc)$_3^-$,

Cu(oAc) $_4^{=}$ and Cu(oAc) $_5^{\equiv}$ is inferred It was not possible to find out the values of β's as it involved complicated system of ten equations with ten unknowns. It is for the first time that I have established the presence of as many as five complexes in this system. The pentacovalency of Cu^{++} is discussed.

Chapter VII *Polarographic Study of the Lead-Glycine Complex.*

The existance of only one complex ion PbG^+ has been shown by polarographic method in lead glycine in—aqueous solutions in three different concentration ranges of glycine at constant ionic strengths of 1 and 1 respectively The results are confirmed by potentiometric and—conductometric titrations.

The dissociation constant of the complex is calculated; the average value being pK=3 49 and agrees with some of the previously reported values. Standard free energy of the formation of the complex was found to be 7·3 kcals.

The formation of the complex ions by lead salts with amino acids has been investigated in the past by several authors. In the earlier works reported, the lead glycine system was studied by methods other than polarographic. There exists certain disagreement also as regards the number of complexes formed and their dissociation constants.

I have, therefore, investigated the above system polarographically in three different analytical concentrations of glycine keeping the ionic strength constant at 1 in the first two series and 1 5 in the third. The results were confirmed by Potentiometric and Conductometric methods

Chapter VIII *Application of polarography in the determination of heavy metals in rocks and the determination of age of igneous rocks*

For the first time I have used the polarographic method for the determination of uranium in rocks Lead was also determined by polarographic method as developed by S. Miholic. Thorium was determined by a microgravimetric method.

The age of two granite samples was determined by knowing the amount (%) of lead, uranium and thorium in these granites. The Aargranite from Switzerland gave an absolute age of 300 million years which tallies well with the relative age determined stratigraphically by Swiss Geologists, while, for the Gotthardgranite, an absolute age of 50 million years was obtained, which corresponds to cretacious or early Tertiary This is for the first time that the age of Gotthardgranite as corresponding to cretacious was reported. This was very well confirmed independently by a different method (Pleochroic haloes) by S Deutsch. The % of uranium is comparatively large. This can be accounted by assuming that the investigated granites are not of igneous origin, but derived from schists that have been granitized and at present there are trends to suppose that all granites have had this origin.

Chapter IX : *Studies in Amalgam Polarography—Polarographic studies with Bismuth Amalgam.*

Amalgam Polarography unlike other branches of polarography has not received sufficient attention as revealed by a survey of the available literature. The tudy of anodic waves in the former has both—theoretical and practical—importance. By collecting data of cathodi and anodic reactions at the electrode under different conditions, t has been possible (I) to verify the fundamental theoretical tatus and the equations in polarography; (ii) to clear our understanding of reversible-irreversible processes occuring at the electrode. From practical point of view studies of anodic waves in amalgam polarogra

phy has opened up the possibility of calculating diffusion coefficients of certain metals in mercury of calculating solubility products of sparingly soluble salts, etc. and in the analysis of many alloys

Filipović first studied bismuth amalgam polarography in details. He found out that in unbuffered alkali chlorides solutions, two anodic waves were obtained whilst buffered—solutions gave rise only to the second but much steeper wave.

Stromberg also studied bismuth amalgam polarographically but his main interest was theoretical, although he has discussed some of the possibilities of the uses of the studies of amalgam polarography

In the present work an attempt has been made to study the electrode processes occurring at bismuth-amalgam electrode in acetate buffered (pH 2·6 to 3 8) alkali-chloride solutions

In the present work an attempt has been made to study the electrode processes occurring at bismuth-amalgam-electrode in acetate buffered alkali chloride solutions. Bismuth amalgam (concentration 1 13 $\times 10^{-4}$ gm. atom/l of mercury) was prepared in a specially prepared glass apparatus. The following equation was derived for the anodic process occurring at the electrode

$$E_{\frac{1}{2}} = \dot{E}a - 0{\cdot}020 \log S - 0{\cdot}040 \log (\overline{OH}) - 0{\cdot}020 \log (\overline{Cl}) - 0{\cdot}020 \log \frac{i - I)}{2k'D_m \frac{1}{2}}$$

from which the following relations were got

$$\frac{\partial E_{\frac{1}{2}}}{\partial pH} = 40 \text{ mvs/pH and}$$

$$\frac{\partial E_{\frac{1}{2}}}{\partial pCl} = 20 \text{ mvs/pCl}$$

Experimental data agree very well with the above derived relations

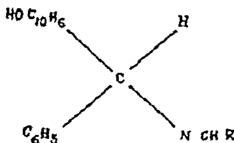
OPTICAL ACTIVITY AND CHEMICAL CONSTITUTION

PART V OPTICAL ACTIVITY AND MOLECULAR FRAMEWORK

O N PERTI

Professor of Chemistry Th D S B Government College Naini Tal

From a discussion of the physical theories of optical activity it follows that in order to obtain information in regard to relationship between structure and rotation it is necessary to compare not the rotation of the molecule as a whole but the partial rotations of the chromophoric group. It was the work of Betti (1901 1906 1907 1916 1920 1930) however which first pointed to the importance of the group which plays a significant role in determining rotatory power He prepared and resolved β-naphthol-phenyl amino methane, a compound containing only one asymmetric carbon atom. He condensed it with a series of aldehydes and produced compounds of the type



(R.CHO is the aldehyde)

A study of such compounds revealed that there is a remarkable parallelism between the molecular rotations of the condensation products and the dissociation constants of the corresponding acids (R.COOH) Some of this data is given in table I

TABLE I

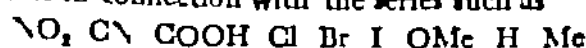
Aldehyde R-CHO	$(M)_D$ of the aldehyde compound	K x 10⁵ at 25 for R-COOH
p-Dimethylamino benzoic	2070°	0 91
p-Oxybenzoic	1040 5	2 9
3-Bromo-p-anisbenzoic	648 0	—
Protocatechuic	580 8	3 3

(Continued on next page)

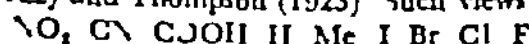
TABLE 1 (*Contd.*)

Aldehyde R·CHO	$(M)_D$ of the aldehyde compound	K×10⁴ at 25 for R.COOH
3–Nitroanisic	559 6	...
m–Toluic	504 5	5 6
Benzoic	373 1	6 6
m–Oxybenzoic	362 6	8 33
p–Chlorobenzoic	311 8	9 3
m–Bromobenzoic	280 9	13 7
m–Chlorobenzoic	255 9	15 5
m–Nitrobenzoic	167 6	31 8
Salicylic	85 7	106
o–Chlorobenzoic	−128 4	132
o–Bromobenzoic	−308 2	145
o–Nitrobenzoic	−990 7	637

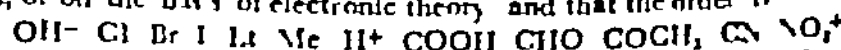
Table I clearly shows that it is the polarity of the substituents which appears to determine the magnitude of molecular asymmetry or optical rotatory power The word polarity as applied to substituent effect is generally used in two senses On the one hand it is used in the phrase general polar effect in connection with the series such as


NO_2 CN COOH Cl Br I OMe H Me

representing the relative influence of groups on the acidic strength of an aliphatic acid This sequence has also been traced in the effect of the substituents on the velocity of reaction and molecular inductive capacity (Rule and Patterson 1924) On the other hand, groups are frequently described as positive or negative either in reference to their directive influence on benzene substitution or deduction from Lapworth's principle (Lapworth, 1920 1922) of induced alternate polarity Lapworth's view has been subsequently developed on the basis of the electronic theory by Kermack and Robinson (1922) and Thompson (1923) Such views led to a series of the type


NO_2 CN COOH H Me I Br Cl F

This series closely corresponds to the relative influence of groups on nitration of benzene Rule (1922) has shown that the arrangement of groups according to their polarities follows a general order with minor differences, whether the order is deduced from benzene substitution data, or influence of various groups on dissociation constants of substituted acetic or benzoic acids, or on the basis of electronic theory and that the order is


OH^- Cl Br I Et Me H^+ COOH CHO $COCH_3$ CN NO_2^+

It was pointed out by Rule (192) that (−)—menthyl esters of monosubstituted acetic acids showed a distinct relationship between the dissociation

constants of the corresponding acids and the optical rotatory power of these condensation products. Even a better relationship is obtained if comparison is made with the values of dipole moments characteristic of the substituent groups. In Table 2 is given some data to illustrate this relationship (Rule, 1930)

TABLE 2

Homogeneous (—)-menthyl esters of monosubstituted acetic acids X $CH_2COO.C_{10}H_{19}$

X—	Dipole moment of X— as $\mu \times 10^{18}$	$[M]^{20°}_{5461}$	Dissociation constant $K \times 10^5$
NEt_2	1.4	−151.6°	small
NMe_2	1.4	150.9	
H	0	157.3	1.8
CH_3	0	160.2	1.4
COOH	0.7 (?)	160.2	160
OCH_3	1.2	165.0	33
OH	1.7	165.0 (at 91°)	15
Br	1.5	169	138
Cl	1.5	171	155
CN	3.8	174	570

These studies led to the view that polar effect is traceable on rotatory power (Betti 1923 Rule, 192 Singh and Bhaduri 1937). It has further been suggested that in general, the replacement of a hydrogen atom in an optically active compound by a positive substituent displaces the rotation in the opposite sense to that due to a negative substituent. Further in general, a positive group should increase the rotation (Singh and Barat, 1940) and a negative group should cause a decrease in rotation (Singh and Bhaduri, 1937)

It is thus seen that the polarity of a substituent group and more fundamentally the partial rotation due to the chromophoric group are of great importance in the study of optical activity in relation to chemical structure of organic compounds. Several types of relationship between optical activity and chemical constitution have been observed. It has also been noticed that the effect of change in constitution in one series is not always the same for a similar change in another series. Moreover frequently it is found that the effect of a group is different in different solvents. There are however certain qualitative regularities which have been observed in different series and which have found use in the determination of molecular structure.

Some of the earlier empirical rules may here be usefully recapitulated in a more precise form. One of these rules is what is known as the Rule of

Superposition (Van t Hoff 1894 Guye and Gautier 1894) According to this rule as proposed originally if there are two or more asymmetric atoms in a compound the optical activity of the individual asymmetric atoms can be added algebraically This is seldom found to be the case because of the mutual effect of different centres on their contribution to the total rotation. Freudenberg s Rule of Shift (*Verschiebungssatz*) which is a qualitative rule is of a more general application (Freudenberg 1933[a]) According to this rule if two similar asymmetric molecules P and Q are altered in the same way to give P and Q then the differences in molecular rotation (P' P) and (Q Q) are of the same sign. In a homologous series containing only one asymmetric carbon atom, Tschugaeff's generalisation (Tschugaeff 1898) sometimes called the Distance Rule (*Entfernungssat*) is important This may be stated as follows In a homologous series containing one assymetric carbon atom, the molecular rotation tends to a limitung value and thereafter shows little alterations (Humphrey and Guye 1903 Guye and Babel 1899)

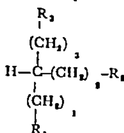
The use of optical rotation in determining structure is based on analogy and not on the physical theories of optical rotation The physical theories are not of much value in dealing with structural problems. For example Kuhn (1935[a]) claimed that the Fischer convention for the formula of glyceraldehyde was correct Wasser (1949) adduced arguments in favour of the other view the arguments of Wasser were discounted by Turner and Lonsdale (1950) and lastly the work of Bijvoet *et al* (1951 [b]) which is now generally accepted as correct shows that the convention proposed by Fischer is the right one

If an adequate series of fair analogies are available then optical rotation can prove to be of great advantage However for any analogy to be rigorous it is necessary that relationships deduced in regard to any group of compounds should be such that the vicinal action of groups involved is either non-existent or negligible. One can only learn about vicinal action from a close study of the rotations of these compounds (Rupe 1914 Rupe *et al*, 1930 1932 1940 Freudenberg and Kuhn 1931 Barton and Cox, 1947 1948) Here again it may be pointed out that correlation of optical rotation of one series with the optical rotation of another series is not desirable as it is seldom found to be correct Moreover other methods are now available which are more useful such as the kinetic method of Ingold (Brewster Hiron Hughes, Ingold and Rao 1950 Ingold, 1953) and the correlation method of Fredga (1944) using quasi racemic compounds.

A mass of optical rotation data is now available and several types of relationship between optical activity and chemical constitution have been observed. These would be considered under the following sub-heads:—

1 Substances having one asymmetric carbon atom
2. Carbohydrates
3 Amino acids, polypeptides and proteins
4 Steroids
5 Terpenoids
6 Others.

I *Substances having one asymmetric carbon atom*—All such substances which have been investigated can be expressed by the general formula



where R_1=CH_3– *iso*–C_3H_7– C_4H_9– or C_6H_{11}–
R_2=CH_3– or a functional group and
R_3=CH_3– and
n_1, n_2, n_3=0 or an integer

Several such series have been investigated and their important results are given below

In optically active hydrocarbons of the type CHR_1R_2R ($R > R_2 > R_1$, *dextro*) Levene and Rothen (1938) found that when the dispersion curve can be expressed by a one term Drude formula the dispersion constant found (λ_0 =1600 to 1700 Å) is nearer to the visible region than the first absorption band of an alkyl group From this they inferred that the rotatory dispersion curve of these hydrocarbons is the resultant of two rotatory components of opposite sign and it is therefore not surprising that when $R_2 > R_1$ the rotation of the hydrocarbon is *laevo*

Secondary carbinols studied earlier by Pickard and Kenyon (1911) were reexamined by Levene and Rothen (1936a b) who extended the measurement of rotatory dispersion to the ultraviolet It was found that the distance of the hydroxyl group from the asymmetric carbon atom, as well as the structure of the alkyl groups had a marked effect on the dispersive power It is interesting to note here that when Pickard and Kenyon (1914) found anomalous dispersion in substances containing only one asymmetric centre they attributed it to a mixture of two isomerides which have rotatory powers of opposite sign and different dispersive powers. Levene and Rothen (1936a b) noticed that the *dext* *s*-octanol-4 (propyl-butyl-carbinol) gave a *laevo* rotation in ether Analysis of dispersion curves disclosed that the dispersion was normal in ether but anomalous in the homogeneous state. This indicated that the first active absorption region of this *dextro*-carbinol furnishes a laevo rotatory partial contribution. Thus the anomalous dispersion observed by Pickard and Kenyon is merely due to the superposition of partial rotations. This explanation is further supported by the work of Kenyon and Barnes (1924) who studied a series of ethers prepared from *dextro*-nonanol-3 (from methyl up to nonyl ether) and found that with the exception of methyl ether all were *dextro*-rotatory The dispersion of methyl ether was normal whereas all others exhibited anomalous dispersion.

The change of rotation with the increase of the distance of the chromophoric group from the asymmetric carbon atom is a very striking phenomenon as noticed earlier by Tschugaeff (1914) and by Rupe (1914) who noted a gradual rise or drop in the rotation of the consecutive members until a constant value was finally reached. The effect of a group on the total molecular rotation is twofold. It introduces a partial rotation of its own and it influences the partial rotations of other groups. The effect of isopropyl group in the case of active hydrocarbons and secondary alcohols has been studied in detail by Levene and Rothen (1936a, b). They found that the effect of the distance of isopropyl group from the asymmetric centre in carbinols is similar to that in hydrocarbons. It was also noticed that the effect of the isopropyl group is exhausted at a comparatively short distance from the asymmetric centre. The effect of phenyl and cyclohexyl groups studied earlier by Haller, Hilditch and Rupe was reexamined by Kuhn and Biller (1935 b) who extended the observations of rotatory power to the ultra violet. These workers carried out an extensive investigation into the effects of substitution of a cyclohexyl or a phenyl group for a hexyl group in nitrites, acetates, carbomethoxy derivatives and phthalates. In all the three series of derivatives namely those of methyl n-hexyl carbinol, methyl cyclohexyl carbinol and methyl phenyl carbinol the shift of rotation on passing from the carbinols to the derivatives is in the same direction. Correlating it with the earlier configurational studies of Levene and Stevens (1930) these authors concluded that the three radicals exerted a similar vicinal effect and that hexyl-cyclohexyl carbinol should be optically inactive. Further the partial rotations of hexyl, cyclohexyl and phenyl groups have the same sign. Here it may be pointed out that the observations limited to a single member of the homologous series cannot be generalised particularly when the active absorption bands are situated in the distant ultraviolet region. Analogy with members of the isopropyl series mentioned earlier would indicate that hexyl-cyclohexyl carbinol should be optically active and *not inactive* as suggested by Kuhn and Biller. This has however not yet been tested experimentally. To sum up one may say that observations on the substitution of an isopropyl for a propyl or of a cyclohexyl or phenyl group for a hexyl indicate that the effects of these substituents are similar.

The influence of ethylenic link was summarized in 1914 by Rupe (1914) in the statement "The presence of unsaturation leads to an *irregularity* in the rotatory effect and not *necessarily* to an increased rotation." Observations of Levene *et al* (Levene and Harris, 1935; Levene and Haller 19[illegible]) on rotations of a number of unsaturated carbinols showed that the rotations of the unsaturated carbinols are opposite in sign to those of saturated carbinols in all cases in which the ethylenic link is situated in the smaller alkyl group but the rotation remains of the *same sign* when the ethylenic bond is situated in the larger alkyl group. In this series it appears that the rotation of the molecule as a whole is associated with the change of the partial rotation of the ethylenic link.

When we consider the influence of chromophoric groups in compounds of the type represented by the general formula given earlier, we find that they are of two types, viz., those in which the configurations of all members of the homologous series with respect to n_4 can be correlated by methods of classical organic chemistry and those whose configurations can only be correlated when $n_4 > 0$. Work on both these types of compounds has been reviewed by Levene and Rothen (1938).

Taking first those compounds in which the configurations of all the members of the homologous series with respect to n_4 can be correlated by methods of classical organic chemistry, it is found that the changes in the direction of the partial rotations with the increase $n = 0$ to $n_4 = 1$ cannot be explained on the basis of any theoretical models advanced for the explanation of the mechanism of optical rotation. In several cases there is observed a periodic change in the shift of rotation in the visible region with the progressive increase in the value of n_4. Sometimes this periodicity is latent and only comes to light when the partial rotations of the chromophoric groups are considered. In the case of aliphatic carboxylic acids, it is found that the rotatory dispersion curves of disubstituted acetic acids can be expressed for all members investigated by a one term Drude formula having dispersion constant as 1850 Å indicating that the first absorption band of the —COOH group at 2050 A displays a negligible Cotton effect. In disubstituted propionic acids (homologous series with respect to n_1) it is found that the first member of the series is dextrorotatory exhibiting anomalous dispersion expressible by a two term Drude formula but the other members have a rotation of opposite sign and display a normal dispersion. Analysis of the data indicates that the sum of two rotatory components of the carboxyl group change sign on passing from the member having $n_4 = 0$ to that having $n_4 = 1$. It is worth noting that when the isopropyl group is substituted in methylpropyl acetic acid for a propyl group the resulting acid has the same sign of rotation but a reverse effect is obtained in similarly substituted propionic acid series. A similar but much accentuated effect is observed in acids containing phenyl group. It appears that in all these cases where the dispersion can be expressed by two terms of opposite sign the first term apparently originates in the carboxyl group. Further the change in sign of rotation from $n_4 = 0$ to $n_4 = 1$ is due to change in sign of the partial rotation of the first contribution. Analysis of rotatory dispersion data in the case of ethylphenyl acetic and propylphenyl propionic acid or 2-cyclohexyl propionic and 3-cyclohexyl butyric acids shows that their rotation in the visible region is mainly determined by the partial rotation of the carboxyl group. In contrast to these acids is the case of mandelic acid (Kuhn and Biller 1935) where the first absorption of the phenyl group is found to be anisotropic and furnishes the major contribution to the rotation of the substance. A similar study in the case of aldehydes was carried out by Hudson, Wolfrom and Lowry (1933) and by Levene and Rothen (1936). In these studies an unmistakable periodic change in the direction of the shift of the partial rotation of the band 2930 Å (the first band of the aldehyde group) was noticed with the progressive increase in the value of n_4.

Considering the case of compounds where the configuration can only be correlated when $n_2 > 0$ we find that most of the work is confined to a study of aliphatic azides and halides From the standpoint of optical rotation generally the azide group $(-N_3)$ behaves like a halogen. The studies of Levene and Rothen (1936b) Kuhn and Biller (1935b) and Levene Rothen and Marker (1936) along with the absorption spectra studies of organic iodides bromides and chlorides show that the assumption that the configurationally related azides and halides always rotate in the same direction is not always tenable.

Attempts have also been made for correlation of configuration in α-substituted carboxylic acids Interest in this field (excluding amino acids) has been mostly confined to azido acids (Kuhn 1930 Kuhn and Braun, 1930) mercapto and sulphonic acids (Levene and Rothen, 1931a) and α-halogen acids (Levene and Rothen, 1934b Kuhn and Braun, 1930 Kuhn, Freudenberg and Wolf 1930) It is found that all halogen acids are configurationally related but the configurational relationship of the azido and of the thio acids cannot be correlated The azido and the halogen group resemble very much and it can be expected that configurationally related halides and azides would rotate in the same direction For some substances this is found to be so but for others it is not valid

Marker (1936) has summarised most of the work mentioned above in a table of precedence of substituents from which it is claimed that the sign of rotations of compounds of the type CHWXYZ can be predicted This important data is given in table 3 in a convenient form.

TABLE 3

Optical rotations of compounds containing only one asymmetric carbon atom

$$
\begin{array}{c}
R_3 \\
| \\
(CH_2)_{n_3} \\
| \\
H-C-(CH_2)_{n_2}-R_2 \\
| \\
(CH_2)_{n_1} \\
| \\
R_1
\end{array}
$$

R_1	R_2	R_3	n_1	n_2	n_3	$[\alpha]_D$	Solvent if any
CH_3	OH	CH_3	0	0	0	+10	
			1			+1	
			2			+1	
			3			+12	
			4			+11	[illegible]
			10			0	
	ON		0			+33	
			1			+72	
						+17	
			3			+13	
			4			+12	[illegible]
			10				

[illegible]

TABLE 3—(Contd.)

Optical rotations of compounds containing only one asymmetric carbon atom

R_1	R_2	R_3	n_1	n_2	n_3	$[M]_D$	Solvent if any
CH_3	COO—C_6H_4—COOH(o—)	CH_3	0	0	0	0	
			1			+88	
			2			+95	
			3			+118	
			4			+128	
			10			+145	Ethanol
	Cl		0			0	
			1			+	
			2			+	
			6			+	Chloroform
	Br		0			0	
			1			+15	Chloroform
			6			+	
	I		0			0	
			1			+59	
			2			+	
			3			+	
			6			+	Chloroform
	NH_2		0			0	
			1			+5	
			2			+	
			6			+	
	OH	COOH	0			−5	Water
			1			−7	
			2			−7	Chloroform
			3			−7	
			4			−8	

(Continued on next page)

TABLE 3—(*Contd.*)

Optical rotations of compounds containing only one asymmetric carbon atom

R_1	R_2	R_3	n_1	n_2	n_3	$(M)_D$	Solvent if any
CH_3	OH	COOH	12	0	0	− 8	[illegible]
		COONa	0			+13	Water
		$COONH_4$	1			+16	
		COOBa	2			+10	
		COONa	3			+18	
			5			+19	
		COOM or COOEt	0			+ 9	
			1			+ 5	
			2	0	0	+ 7	
			3			+	
			12			−10	Chloroform
		$CONH_2$	0			+20	
		CHO	3			+33	Ethanol
	Cl	COOH	3			+16	Ether
			4			+	
		COOM	0			+ 4	
	Br	COOH	1			+57	
			3			+74	
		COOM	0			+92	
	NH	COOH	0			− 2	Water
			1			− 8	
			2			− 6	
			3			− 8	
			0			−13	Acid
			1			−20	
			2			−27	
			3			−28	
			9			−5	Glacial acetic acid

(Continued on next page)

TABLE 3—(*Contd*)

Optical rotations of compounds containing only one asymmetric carbon atom

R_1	R_2	R_3	n_1	2	3	$(M)_D$	Solvent If any
CH	CH_3	COOH	0	0	0	0	
			1			−18	
			2			−21	
			9			−13	Chloroform
			23			−28	
	OH	CH_3	0		1	−	
			1			0	
			2			+5	
			3			+9	
			4			+11	
			14			+13	Chloroform
	OAc		0			−	
			1			0	
			2			−1	
			3			−7	
			4			−7	
			14			−9	Chloroform
	COO— C_6H_4 COOH(o—)		0			−	
						0	Ethanol
			1			+19	
			2			+52	
			3			+60	
			4			+63	
			14			−	
	CH		0			0	
			1			+10	
			2				

(Continued on next page)

TABLE 3—(*Contd*)

Optical rotations of compounds containing only one asymmetric carbon atom

R_1	R_2	R_3	n_1	n_2	n_3	$(M)_D$	Solvent if any
CH_3	CH_3	CH_3	3	0	1	+14	
			4			+18	
	OH	COOH	0			−26	Water
			1			−12	
		COONa	1			− 8	
	Cl	COOH	0			−61	
	NH_2					−40	
	CH_3					0	
			1			−19	
			2			+4	
			3			+7	
			4			+12	
			14			+14	Chloroform
C_6H	OH	CH	0		0	+7	
	OAc					−5	
	COO— | C_6H_4 | COOH(o−)					+150	Ethanol
C_6H_5	OH					−52	
	OAc					−199	
	COO— | C_6H_4 | COOH(o—)					+110	Ethanol
	OH		1			+33	
	OA					+11	
	COO— | C_6H_4 | COOH(o—)					+77	Ethanol

(Continued on next page)

TABLE 3—(Contd.)

Optical rotation of compounds containing only one asymmetric carbon atom

R_1	R_2	R_3	n_1	n_2	n_3	$(M)_D$	Solvent if any
C_6H	OEt or OMe	CH_3	0	0	0	+4	
C_6H_5						−70	
			1			+38	(M)[illegible]
	Cl		0			−71	Ethanol
			1			+22	Ethanol (M)[illegible]
	Br		0			−	Ethanol
			1			+13	Ethanol (M)[illegible]
C_6H	NH_2 HCl		0	0	0	+	Ethanol
C_6H_5	NH_2					−37	
			1			+39	
C_6H	OH	COOH	0			−42	Water
		COONa or COOK				+13	
C_6H_5		COOH				−232	
		COONa or COOK				−218	
		COOH	1			+63	
		COONa or COOK				+150	
C_6H_5	Cl	COOH	0			−325	
			1			+	
	Br		0			−302	
			1			+	
	NH		0			−170	Water
						−238	Acid
			1			+58	W ter
						+12	Acid
C_6H_{11}	OH		0			−26	

(Continued on next page)

TABLE 3—(*Contd.*)

Optical rotations of compounds containing only one asymmetric carbon atom

R_1	R_2	R_3	n_1	n_2	n_3	$[M]_D$	Solvent if any
C_6H_5	CH_3	COOH	0	0	0	−112	
COOH	OH		1			+4	Methanol
	Cl					+72	Ether
	Br					+139	Ethyl acetate
	NH_2					+16	Water
						+32	Acid
	CH_3					+12	Water
COOMe	OH	COOMe	1	0	0	−11	
	Cl					+16	
	Br					+146	
COOH	CH_3	COOH	2	0	1	+15	Water
	OMe					+25	Chloroform
CH_3	OH	OH	0	0	1	−8	Ethanol
"			1			+11	
			2			+16	
			3			+18	
	CH_3		1			+5	
		Br				−8	
OH	OH	COOH	1		0	−2	Water
		COOCa				+17	
Br		COOH				−3	
NH_2						+33	

As an example of the application of the data given in table 3 may be cited the case of *anteiso* acids which have been obtained from wool wax and mutton fat (Weitkamp 1945, Hansen, Shorland and Cook 1952, 1953). Synthetic studies of Velick and English (1945), Crombie and Harper (1950) and Stallberg Stenhagen (1950) were based on (−)−L−methyl butanol and showed that the three members of the L-methyl series all have positive rotations of the same order of magnitude. This led Crombie and Harper (1950) to suggest that the other (+)−*anteiso* acids belong to L-series.

2. *Carbohydrates*—Optical rotation of carbohydrates and its correlation with their structure has been largely studied by C S Hudson and co-workers. The collected papers of Hudson have been published in two volumes by Academic Press New York, in 1946 Most of the data on optical rotatory power of carbohydrates has been tabulated by Bates *et al* (1942)

Hudson (1909) found that the rotations of simple aldoses and their glycosides could be analysed on the principle of optical superposition. This he stated in the form of two rules of iso-rotation (1) "The difference between the molecular rotations of the α and β-forms of all the aldehyde sugars and of all their derivatives in which the added substance is not joined directly to the end asymmetric carbon atom is nearly a constant quantity and, (2) The α- and β-forms of these derivatives (*e g*., glucosides etc) of any aldose sugar in which only the end asymmetric carbon is affected have molecular rotations whose sum is equal to the sum for the α- and β- aldoses" Molecular rotations of some monosaccharides disaccharides and their derivatives have been tabulated and discussed by Klyne (1951)

Hudson (1930 a b) found that the rotational magnitudes divided the sugars into two or three categories He assumed that these categories must correspond to differences in chemical structure—a claim which was hotly controverted by Haworth and Hirst (1930 *et seq*) on the basis of methylation and degradation studies. This Hudson-Haworth controversy has been very well discussed by Hirst in Hudson Memorial Lecture to the Chemical Society" (June, 1954) It may be mentioned that later work confirmed the views of Haworth and Hirst, even by the work of Hudson's colleagues when they applied the periodate oxidation method for evidence regarding size. It is worthwhile to quote from Haworth and Hirst's paper the caution needed against arbitrary assumption in regard to optical rotatory power and chemical constitution: "It seems to us to be reasonable to test a hypothesis and the deductions made from it by comparing them with experimental results but when additional and entirely unproven assumptions are superimposed on the first to account for discrepancies which arise between the deductions and the observed facts (rotational values) the additional assumptions can have no assurance of validity

The configurations of asymmetric carbon atoms in the common glycosides is generally given on the basis of evidence of periodic acid oxidation (Jackson and Hudson 1937) However in the case of glycosides of rare sugars such as deoxy sugars studied by Reichstein, Stacey *et al* the rotations have been found to be useful to indicate the configuration of anomeric carbon atom. Study of optical rotations of synthetic steriod glycopyranosides indicates that the carbohydrate contribution ΔG is very approximately equal to $(M)_D$ of the corresponding α- or β-methyl glycopyranoside. This suggests that the rotational contribution of the carbohydrate component is almost independent of the nature of the steriod component (Klyne, 1950) It is, therefore, plausible to use rotation differences to indicate the configuration at

the anomeric carbon atom in steriod glycosides if the $(M)_D$ values of the corresponding methyl glycosides are known. Most of the synthetic glycosides known are β-anomers but in the case of cholestanyl glycosides and their acetates both α and β-anomers are available (Linstead, 1940). In table 4 are given the configurations of the anomeric carbon atoms in cardiac glycosides containing one molecule of sugar. The table has been compiled by Klyne based partly on his work (Klyne, 1950) and that of Reichstein and his colleagues (Reichstein *et al* 1951) during 1950-54.

TABLE 4

Configuration of anomeric carbon atom.

Name of carbohydrate	*Configuration of anomeric carbon*
D—Cymarose	β—
D—6-Deoxyallose	β—
D—Diginose	β—
D—Digitalose	β—
D—Digitoxose	β—
L—Rhamnose	α—
D—Sarmentose	β—
D—Thevetose	β—
L—Thevetose	α—

Mention be also made of the ethers of 2 hydroxy tetrahydropyran studied by Woods and Kramer (1947) and by Patham and Anderson (1948). They are like hemiacetal pyranoside minus the alcoholic groups. When 2, 3-dihydropyran is treated with alcohol in presence of hydrochloric acid, the resulting hemi-acetals are analogous to steriod derivatives prepared by Greenhalgh, Henbest and Jones (1951). Their configurations can be correlated on the basis of optical rotation magnitudes.

Hudson also suggested the application of the rule of shift of rotation in indicating the type of glycosidic linkage between the two components of a disaccharide (Hudson 1916). This work was extended by Freudenberg, Friedrich and Baumann (1932) to poly saccharides like starch, cellulose and their degradation products. According to them if any polysaccharide consists of n similar unit the molecular rotation $(M)_n$ can be expressed as the contribution of the first (a) intermediate (m) and terminal unit (z)

$$(M)_n = a + (n-2)\, m + z$$

From such a relation it is obvious that when n is very large (M)' repeating unit m Freudenberg, Kuhn, Durr, Bolz and Steinbrunn 1930, Meyer, Hopff and Mark, 1930). A review of the available data indicates that in case of polysaccharides with one type of repeating unit and one type of coupling the α linkages would show a large positive value for molecular rotation (~ +400) and the β-linkages a very low value approximately

zero (Freudenberg, 1933 b Bailey Whelan and Peat 1950) It is on this basis that Hirst and Jones (1946) have suggested mainly β linkages for the polygalacto-pyranose obtained from seeds of *Lupinus albus* for which $(M)_D$ /unit $= -26$ and mainly α linkages for pectic acid (a poly D-galacturonic acid) for which $(M)_D$ /unit $= +490$

For configuration at C_2 carbon atom Hudson's amide rule (Freudenberg and Kuhn 1931 Clough 1915 1918 Hudson 1918 Hudson and Komatsu, 1919) or the related hydrazide rule (Hudson, 1917) are useful According to these rules the Δ—value for CONH —COOH that is, Δ—amide or Δ—hydrazide (i e CO.NH.NH C_6H_5—COOH) is positive if C_2 hydroxyl has D-configuration and negative if it has L-configuration For example the Δ—amide values in the case of L-mannonic acid (+33) D-gulonic acid (+41) D-α-glucoheptonic acid (+48) D ribonic acid (+56) L-talonic acid (+66) L-arabonic acid (+79) D xylonic acid (+76) D-gluconic acid (+74) and D-galactonic acid (+83) are all positive indicating that C_2 hydroxyl has D-configuration

Kuhn pointed out (Freudenberg 1933a) that the configuration of the C_3 influences the magnitude of the Δ—amide value in respect to C_2 but the configurational differences at or beyond C_4 have practically negligible influence. In the simpler case of 2 D-hydroxy acids Levene 1915) noticed that salts were more dextrorotatory than the free acid and a converse effect was found in the case of 2 L-hydroxy acids. Mention be also made of the work of Deulofen (1933) on acetylated nitriles of sugar acids. He found that 2 D and 2 L acetoxy nitriles have positive and negative rotations, respectively By analogy Schmidt (1950) deduced that the configuration of the hydroxyl group at C_2 in apionic acid was D

The configurations at C_4 and C_5 involve ring formation which greatly effects the optical rotation Here Hudson's Lactone Rule (Hudson 1910 1939a) provided useful information. According to this rule if the configuration of the hydroxyl group which takes part in γ- or δ-lactone formation is D the lactone has a positive rotation if L the lacton has a negative rotation This rule is not universally applicable as in certain cases such as that of D-allonic-γ lactone (—12) the effect of other hydroxyl groups may over balance the effect of the lactone C atom. The lactone rule has, however been extensively used in carbohydrates to deduce the configuration of hydroxyl groups Some of the important applications are pyranose structure of glucose (Pryde 1923) configuration in rhamnose (Jackson and Hudson 1930) and fucose (Hudson, 1911 Clark, 1922) The application of lactone rule has also been extended to polycyclic compounds (Klyne 1951)

Pacsu (1939) pointed out the possibility that differences in conformations might effect rotational calculation in mannose and sorbose series. Similar suggestions have also been given by Hudson (1939) The conformation of pyranose rings have been discussed by Hassel and Otter in the light of their concept of K and E bonds generally called 'equatorial' and

axial by British and Americans (Beckett, Pitzer and Spitzer 1937 Barton, Hassel Pitzer and Prelog 1953 1954) Reeves (1950) studied this problem using copper ammonium salt complex method and found that nearly all D-glycopyranosides have what he calls C-1 chair conformation and L-glycopyranosides have the 1-C conformation—a result which is in general agreement with the suggestions of Hassel and Otter There are however certain exceptions (D—iodose derivatives are 1 C D-altose and D-lyxose derivatives are mixtures of 1-C and C-1) which go to show the possibility of some further complicating factor over and above the vicinal effect of the neighbouring groups. It may also be mentioned that amylose appears to contain two types of boat form glycopyranoside residues (Reeves, 1954)

In the case of sugars inorganic complexing reagents have been widely used to increase the magnitude of rotation. The inorganic complexing reagents which have been used extensively for this purpose are boric acid (Fischer 1890) molybdic acid (Gernez 1891 Schmidt and Weber Molster 1931 Bennet-Clark, 1934 Hudson 1916) and copper ammonium salt solution (Reeves, 1951)

3 *Amino acids polypeptides and proteins*— Optical rotation data has been used for two purposes in the case of amino acids. On the one hand it has been used to determine stereochemical relationship of α-amino acids with α hydroxy acids with a view to correlate it with glyceraldehyde. On the other hand it has been used to determine the stereochemical relationships of different amino acids

Freudenberg and co-workers (Freudenberg and Rhino 1924 Freudenberg and Meister 1935) made a comparison of optical rotations of compounds of the type $CH_3 CH(OR) COR$ and $CH_3.CH(NHR) COR$ Though there were several exceptions the rotation values generally tended to show that (+)-alanine isolated from hydrolysis products of proteins and peptides was configurationally related t L-(+)-lactic acid. Their evidence (Freudenberg Kuhn and Baumann 1930) based on comparison of rotatory power of derivatives of α azido p opionic a ids and α halogen propionic acids wa much more satisfactory if one k pt in mind that azido acids can be reduced to amino acids (Cowdrey Hughes and Ingold 1937; Cowdrey Hughes Ingold Mastermann and Scott, 1937 Amino acids can also b configurationally related to sugars involving arguments from D-glucosam ne which resembles D-amino acids in optical behaviour (Levene 1925 Levene Mori and Mikeska 1927 Levene Bass Rothen and St ger 1929 Karrer and Jirgensons 1930, 1931 1932) Neuberger 1948 h s discu ed a series of arguments involving D glucosamine and the degradation of D-gl icosamine to alanine by Wolfrom, Lemieux and Olin (1949) when t ken together confirm the views of Freudenberg et al The X ray work in th onine (Shoemaker Donohue Shomaker and Corey 1950) and hydroxy pr l n (Zussmann 1951) and arguments bas d on react on mechanisms (Brewster Hron Hughes Ingold and Rao 1950 Ingold, 1951) further provide confi m tory evidence

The rotatory dispersion studies also point to a similar configurational relationship. Lifschitz (1925) observed that the copper complex of L-(+)-alanine shows anomalous rotatory dispersion in the visible range of the spectrum. This work was later extended by others (Pfeiffer and Christeleit, 1937 a, b; Karrer and Meyer 1937) to copper complexes in a series of α-amino acids. It was observed that with the exception of copper complex of L-( )-proline the amino acids of like configuration at α-asymmetric carbon atom show Cotton effect curves of a like sign with an extremum near 550 ± 10 mμ. It was found that the Cotton effect curves of copper comlexes of L-(+)-alanine and L-(+)-lactic acid are positive (Pfeiffer and Christeleit 1937b). Even though the Cotton effect curve of L-(+)-lactic acid has an extremely small amplitude and has to be measured in concentrated solution to avoid dissociation of copper complex, the general agreement with the curve of L-(+)-alanine was quite clear. A much more suitable comparison is found when α-hydroxy acid alkyl dithiocarbonates (xanthates) are compared with the corresponding α-amino acid alkyl thiol thio carbamates (urethane). These derivatives have been used in the quasi-racemate method of Fredga (1941, 1942) and the rotatory dispersion studies by Sjoberg, Fredga and Djerassi (1959). The rotatory dispersion studies indicated that the sign of the Cotton effect curve of the α-amino acid dithio carbamate corresponds to the sign of the Cotton effect curve of the configurationally related α-hydroxy acid xanthate. Moreover the dithiocarbamates of α-amino acids show strong Cotton effect curves whose sign can be related to the configuration of the α-carbon atom.

It is fortunate that the initial tentative suggestions of Freudenberg *et al* regarding the stereochemical configurational relationship of L-(+)-alanine and L-(+)-lactic acid was confirmed by later workers using different type of arguments. This should not lead to the view that the method based on optical rotation alone can provide unambiguous evidence in correlating one series of compounds with another. It may be mentioned that similar arguments were used by Levene *et al* (Levene 1925, Levene, Mori and Mikeska 1927, Levene, Bass, Rothen and Steiger 1929) and by Clough (1915, 1918) to assign D-configuration to (−)-α-halogen substituted carboxylic acids because they showed a positive Δ-value on salt formation. Subsequent studies based on kinetics showed that they had L-configuration (Ingold 1953).

The interrelation between optical rotation and spatial configuration of different amino acids have been studied in several different ways. The first of these studies were those of Clough (1918) extended by Freudenberg (Freudenberg and Meister 1935) and given a semitheoretical basis by Kuhn (Freudenberg, 1933). These studies involved the changes in optical rotation on formation of hydantoins and similar cyclic derivatives. It was found that all amino acids showed a negative Δ-value for hydantoin-neutral amino acid, a conclusion which pointed to the L-configuration of natural amino acids of animal proteins (Neu 1948). Secondly was the correlation of rotatory

dispersion and amino acid configuration by Karrer and Kaase (1919) which was later discredited by Waser (1923). Thirdly was the work of Lutz and Jirgensons (1930, 1931) dealing with the effect of pH on optical rotation of amino acids. It was found that all amino acids except isovaline showed a positive Δ-value for cation—neutral molecule. Mention be next made of the rotatory dispersion studies of Patterson and Brode (1943). Based on the study of 14 different amino acids using various conditions of pH and measuring rotations over the range 660 to 440 mμ they concluded that in the case of amino acids one of the following conditions are observed (1) the dispersion is normal positive and intercepts the zero axis in the region of the spectrum above 205 mμ (2) the dispersion is normal negative and intercepts the zero axis in the region below 140 mμ squared (3) the dispersion is anomalous and the sign of rotation changes from negative to positive with decreasing wavelength. The rotatory dispersion studies were later extended by Otey, Greenstein, Winitz and Birnbaum (1955) and by Djerassi (1960). Following Djerassi if we define a chromophore as a group that has a well characterised absorption band in the Schumann region or above we find that for amino acid with no chromophore beyond the α-carbon atom, for amino acids with β-chromophores and for amino acids with chromophores beyond the β-carbon atom the dispersion measurements near 200 mμ represent a means of giving prominence to the —COOH band and give similar results as the acid shift studied by Lutz and Jirgensons. The rotatory dispersion studies in these cases can be summed up in a simple rule as given by Djerassi. L-amino acids with no or weak chromophores on the β-carbon atom give plain positive [illegible] curves; amino acids with powerful chromophores on the β-carbon atom [illegible] anomalies in the near ultraviolet or visible but conform at lower wavelengths. On the other hand in the case of amino acids with more than one asymmetric centre (threonine, isoleucine, phenyl-serine and amino tricarballylic acid), cyclic amino acids (proline and hydroxy-proline) and cystine the dispersion measurements show a very different character of rotation and the observed positive acid shift for the L-form appears to be only a chance agreement. In these cases it is not possible to arrive at any generalisation.

T[illegible] [illegible] acids were studied by Pertzoff (1937) and have been described [illegible] uberger (1948).

Wh[illegible] [illegible] made to apply the generalisations obtained in the case of ami[illegible] [illegible] peptides it were largely unsuccessful. It is only in rece[illegible] [illegible] that methods have been made available for the synthe[illegible] of polypeptides [illegible] molecular weight (Blout and [illegible] 195[illegible] [illegible] and Blo[illegible] [illegible] Des Roches, 1959; [illegible] and Sela, 19[illegible]) and investigat[illegible] [illegible] rotatory power has been undertaken. It has been observed that the [illegible] factors relating to amino acid the conformation of th[illegible] [illegible] has the nature of the side chain attached to the β-carbon atom [illegible] amino acid and the length of the peptide chain influence the [illegible] rotatory power in synthetic polypeptides.

A polypeptide chain in which the component amino acid residues have no periodic internal structure is defined as being in the random coil or the random conformation. In such a case their rotatory properties are simple and their dispersion can be described by a one term Drude equation e g., poly-1-benzyl-L-histidine and poly-γ-benzyl-L-glutamate (Doty and Yang 1956) Following the suggestion of Pauling and Corey (1950) that the α-helix structure—a totally intramolecularly bonded structure—is of importance in synthetic polypeptides and proteins, Cohen (1955) indicated that changes in optical rotation that accompany the denaturation or unfolding of protein may be due to changes from helical structure to a more random conformation. Moffitt (1956 b) and Moffitt and Yang (1956) mad the suggestion that such helices might behave optically as a single absorbing system (an exciton system) and derived a phenomenological equation which was later modified by Moffitt, Fitts and Kirkwood (1957) When polypeptides are in α-helical conformation such as poly-L-alanine, poly-L-lysine poly-L-α-amino-n-butyric acid (Downie Elliot Hanby and Malcom 1957) poly-γ-benzyl-L-glutamate (Doty and Lundberg 1957 Yang and Doty 1957) poly-L-glutamic acid (Idelson and Blout, 1958) poly-L-lysine and poly-L-carbobenzoxy-L-lysine (Applequist, 1958) the rotatory dispersion may be described by Moffitt equation and it is possible to estimate the helix content Such a type of analysis does not appear to be applicable to poly-β-benzyl-L-aspartate (Blout and Carlson 1958) poly-L-proline (Berger Kurtz and Katchalski 1954 Kurtz, Berger and Katchalski, 1956 Steinberg, Berger and Katchalski 1958a b) poly-o-acetyl-L-hydroxy proline poly-L-hydroxy proline (Kurtz, Fasman, Berger and Katchalski, 1958) poly-L-tyrosine poly-o-acetyl-L-serine, poly-1-benzyl-L-histidine, poly-L-serine poly-L-histidine and poly-1-benzyl-L-histidine salt (Djerassi, 1960) Harrington and Sella (1958) are trying to analyse the data regarding proline containing polypeptide.

For lower peptides Brand and coworkers (Erlanger and Brand 1951 b,a) Brand Erlanger and Sachs, 1952 b Sachs and Brand 1953 1954) noted that optical rotation is affected by the chain length in molecules (2-6 peptide units) There is very little doubt that in polypeptides as well as in proteins there may be helical or random conformation or both together There are other possibilities also such as β- non helical or non random conformation Robinson and Bott (1951) showed that optical rotation of a polypeptide series of various molecular weights did not change when the solvent was one that induced the random conformation of the polypeptide thereby indicating that in a random conformation the length of chain beyond a certain size will probably have little effect on the optical rotatory power In the case of helical conformation the basic unit is six peptide links and there is evidence to indicate that helices may be formed in organic solvents when the chain length is only slightly above six such residues (Idelson and Blout 1957) Here therefore the question of chain length becomes quite important in all considerations of optical rotation data (Schellmann and Schellmann 1958) It is worth mentioning that helix stability may be lower in aqueous media

than in organic solvents (Schellmann 1955). The "β" conformation (i. e., twofold screw) of a polypeptide gives rise to different optical properties as compared with helical conformation as first demonstrated by infra-red spectroscopic measurements (Ambrose and Elliot, 1951 Blout and Asadourian 1956). In the case of a low molecular weight polypeptide of poly benzyl-L-glutamate (Yang and Doty 1957) it was found that in high concentrations in chloroform the positive rotation increased with diminishing wavelength and at low concentrations the rotations became more negative with decreasing wavelengths. This indicated a non helical non random conformation where the optical behaviour is probably due to *inter* molecular hydrogen bonding which is the basis of dissymmetry giving rise to optical rotatory power.

Great caution is necessary in such studies as there is evidence to indicate that polypeptides may change conformation in different solvents (Doty and Yang 1956) and at different temperatures (Doty Wada, Yang and Blout, 1957). According to Schellmann (1958) inverted transitions can occur only in solvent mixtures and then only when the entropy of unfolding changes sign.

The polypeptides containing proline and hydroxyproline which are not truly amino acids but imino acids may also be mentioned here. Poly-L-proline (Berger Kurtz and Katchalski 1954) when prepared fresh showed a small positive rotation but in acetic acid after 100 hours it showed a strong negative rotation of $-540°$ (Kurtz Berger and Katchalski 1956 Steinberg, Berger and Katchalski 1958). This mutarotation was explained as probably involving *cis→trans* change around the peptide link. Studies of Blout and Fasman (1958) indicate that poly-L-proline as originally obtained possesses neither random nor α-helical conformation.

The data available at present in regard to optical rotatory power of peptides is very meagre and it is yet too early to arrive at any useful generalisations in regard to their conformation chain lengths cross linkages and so on.

Th optical rotatory power of proteins presents still more complex problems. The number and kind of amino acids and their sequence in the protein molecule are bound to effect the rotatory power. Moreover the molecular wei ht of protein and its conformation are likely to be important factors. Som of the results so far obtained are briefly described below.

The rotatory dispersion of many proteins can be expected by a one term Drud equ tion and th di persion constant is found to ary systema tically with the extent of the folding (Linderstrom Lang and Schellmann 1954). The d spers on co tant in aqueous sol ition appe rs to ind cate the helix content of prote s [illegible] and Doty 1957). Jirgensons (1957 19 [illegible]) Jirgensons and Straumanis 1957) has clas fied proteins int three groups

according to their rotatory power *viz.*, (i) those which show decrease in the dispersion constant upon ionisation and denaturation (ii) those which show practically very little change of dispersion constant upon denaturation and (iii) proteins whose dispersion constant rises upon denaturation. Perlmann's detailed investigation (Perlmann, 1959) of pepsin in aqueous solution showed an increase of dispersion constant under the influence of denaturing agents Cohen and Szent-Gyorgyi (1957 1959) studied fibrous proteins by x-ray diffraction method and have made the suggestion that these fibrous α-proteins may consist of cables of α helices in a supercoil conformation or coiled coils (Crick, 1953 Pauling and Corey 1953) The recently investigated fibrous protein Pinna tropomyosin (Kay and Bailey 1959) is found to be similar in optical behaviour as early investigated fibrous proteins. Both collagen and gelatin are found to show a rotatory dispersion which can be expressed by Drude's one term equation (Cohen 1955) The effect of temperature on rotatory power of proteins has also been studied (Schellman 1958 Simpson and Kazmann, 1953 Jirgensons 1958[a, d]) and it has been found that the temperature dependence of protein conformation is very complex. Solvent as pointed out in the case of polypeptides, has also profound influence as it can change the conformation from helix→random (Harrington and Schellman, 1956 Doty 1959)

It is yet too early to say anything definite about the correlation of conformation with optical rotatory power in the case of proteins. All that can be safely said is that it appears possible that rotatory dispersion measurements may possibly throw light on general conformations in proteins

4 *Steroids*—The correlation of optical activity and chemical constitution has proved to be of great significance in the field of steroids where we have a fairly flat and rigid molecule and, where in many compounds it is found that functional groups are widely separated and are generally free from vicinal action The early work in this field is due to Callow and Young (1936) and to Lettre (1936) This was followed by studies of Bernstein *et al* (Bernstein Kauzmann and Wallis 1941 Bernstein, Wilson and Wallis, 1942 Bernstein, Hicks Clark and Wallis, 1946) and Barton and Collaborators (Barton 1945 1946 & 1949) It was found that the principle of optical superposition is very much applicable in the field of steroids and their derivatives. Barton has given the Method of Molecular Rotation Differences which is a slight variant of the general rule of shift. In the case of steroids it is generally found that the molecular rotation of a steroid is the sum of the rotation of the fundamental structure (androstane, pregnane or cholestane) and the rotational contributions of the functional groups (usually called Δ-values) The rotational contribution of the functional groups are generally found to be independent of one another (Barton and Jones 1944 Barton and Klyne 1948) but in certain cases where either the groups are placed in close proximity or there are highly unsaturated groups present in the steroid the vicinal action cannot be neglected. Barton and Cox (1947

1948) have summarised their findings about vicinal action Substituent groups may be divided into three classes (i) Those causing no anomalies in the molecular rotation, *i. e.*, C–H C–C, C–OH, –O– groups which are not easily polarised and which do not distort the molecular framework. (ii) Those causing major anomalies only in presence of class (iii) substituents *i. e.*, C–Br C–OAc, C–OBz C=O C=C groups which are more or less easily polarised but which do not cause a high degree of strain in the molecular framework. (iii) Those causing in general major anomalies if another member of class (ii) or (iii) is present, *i.e.* C=C, C=C–C=O C=C–C=C groups which are easily polarised and which distort the molecular framework. Clearly there is no rigid dividing line between the groups placed in classes (ii) or (iii) and for example the ethylenic linkage must be placed in both of these classes It has further been found that there is a rough correlation between the optical anomaly the number of saturated carbon atoms between the two substituents and the absorption maxima in the farthest ultraviolet characteristic of the two substituents In several cases the optical rotation data has been found to be useful in locating the position of a group or substituent in the steroid molecule. In α–spinasterol the position of the nuclear bond was thought to be at C_8–C_{14} (Fernholz and Rugh, 1940) or at C_8–C_9 (Stavely and Bollenback, 1943) but optical rotation values indicated the location at C_7–C_8 which was later confirmed by synthetic studies (Barton and Cox, 1948[a, b] Fieser Fieser and Chakravarti 1949) The configuration of hydroxyl at C_{20} in the case of pregnane–3α 17α–20–triol (Butler and Marrian 1937 1938) was shown to be α– by Klyne (1949) who compared it with O and J adrenal substances of Reichstein (Steiger and Reichstein 1938) The steroid alkaloid rubijervine was shown by Sato and Jacobs (1949) to have the additional –OH at 12–α– position From the urine of pregnant mares a hydroxy ketone was obtained which was found to be androstan 3β ol–x–one (Heard and McKay 1936) It was found to be not identical with androstan–3β ol 7 one (Heard and McKay 1946) and optical rotations indicated either position 15 or 16 for the keto group Later it was found to be 16 (x) (Huffman and Lott 1951)

A detailed examination of the rotatory dispersion curves in the steroids has given fruitful results. Djerassi *et al* and others have examined from 1955 onwards a number of saturated steroid ketones and aldehydes 'Saturated' here mean those steroids in which there is no double bond in close proximity to the carbonyl group The result of such studies (Djerassi Closson and Lippmann 1956 Foltz Lippmann and Djerassi 1955 Djerassi and Closson 1956 Djerassi and Ehrlich 1956 Djerassi Riniker and Riniker 1956 Pelletier and Locke 1957 Djerassi, 1957 Djerassi Ourisson Riniker and Riniker 1958 Djerassi Halpern Halpern Schindler and Tamm 1958 Djerassi Halpern Halpern and Riniker 1958 Schindler Tamm and Reichstein 1958 ... Desaulles Fischer Wieland and Wettstein ... Bowers Ringold ... Denot 1958 Rapala and Farkas 1958 ... Schindler Tamm and Reichstein 1959 Nussbaum Popper Oliveto Friedman

and Wender 1959 Beal Rehenstorf and Pike 1959) indicated that in the ultraviolet the rotation values of a $>C=O$ containing substance are invariably greater than those of the corresponding compound not having this group. This is due to the Cotton effect. Better differences are obtainable in the ultraviolet region where the contrasts are more marked. In such compounds the rotatory dispersion data offers excellent means in locating the carbonyl group. In the case of the alkaloid rubijervine mentioned earlier the rotatory dispersion data of the derived ketone indicated it to be a 12 keto steroid thereby indicating that one of the hydroxyl group is at position 12 (Pelletier and Locke 1957) Reichstein and collaborators used with advantage the rotatory dispersion curves of steroidal aldehydes (Djerassi Halpern Halpern, Schindler and Tamm 1958) to narrow down the structural possibilities of bufotalinin (Schroter Tamm and Reichstein, 1958) and pachygenin (Schmid, Uehlinger Tamm and Reichstein 1959) The correlation of the Cotton effect curves of the 20-keto group in pregnane derivatives (Foltz, Lippmann and Djerassi 1955 Djerassi, 1957 Djerassi, Halpern Halpern Schindler and Tamm, 1958) with the 17-β oriented side chain has been used with advantage in locating the carbonyl group in 5α-pregnane-3β 16α-diol-20-one (Nehr Desaulles Vischer Wieland and Wettstein, 1958) Djerassi (1959) has fully reviewed examples of the application of rotatory dispersion data in settling such stereochemical problems

In the case of α β-unsaturated steriod ketones it is found that the low intensity long wavelength band (above 300 mμ) is very sensitive to the polarity of the solvent employed (Cookson and Dandegaonker 1955) Djerassi, Riniker and Riniker (1956b) examined the effect of three solvents—methanol, dioxan and octane—on the rotatory dispersion curve in the case of Δ^4-cholesten-3-one. They found that the polarity of the solvent has the same effect upon the rotatory dispersion picture as upon the ultraviolet absorption. In most of the rotatory dispersion work in this field the most suitable solvent has been found to be dioxan In these compounds it is found that on the whole the nature of the angular substituent is of no particular significance but its orientation governs the sign of the Cotton effect curve. This enabled the assigning of α-orientation to the angular hydrogen atom at C-5 in the case of 19-nor-$\Delta^{1(10)}$-androsten-17β-ol-2-one (Fishman 1958) by comparing its rotatory dispersion curve with that of 19-nor-testosterone (Pederson et al 1956 Perez Irarte Kincl and Djerassi 1958) Similar arguments were used in deciding the configuration at C-10 in the case of 19-nor-14β 17α-progesterone (Barber and Ehrenstein, 1957) In the case of steriodal diketones (Djerassi and Closson, 1956) sufficient data has not yet been collected to arrive at useful generalisations.

The rotatory dispersion studies have also been extended to bicyclic and tricyclic ketones (Djerassi Riniker and Riniker 1956a Djerassi and Klyne, 1956) It has been found that qualitatively speaking the characteristic rotatory dispersion picture is governed by the spatial environment (. structural

stereochemical and conformational) surrounding the carbonyl group. Hence it is possible to compare analogous structures to decide about spatial arrangement. By comparing the rotatory dispersion curve of des-A-$\Delta^{9(11)}$-lumisten-5-one, a degradation product of lumisterol (Castells Jones Meakins and Williams 1959) with 4β-methyl-coprastan 3-one (Djerassi Halpern, Halpern and Riniker 1958) it is possible to provide evidence for the stereochemistry at C-9 The stereochemical configuration of the four reduction products of (–)-a-santonin were suggested by Djerassi, Riniker and Riniker (1956a) on the basis of rotatory dispersion analysis which were subsequently found to be correct (Yanagita and Futaki 1956 Yanagita and Ogura, 1957 Cocker and McMurry 1958) The two hydrogenation products of β-nor-cholesterol were assigned certain conformation of rings A/B (Dauben and Fonken 1956) and rotatory dispersion studies (Djerassi, Marshall and Nakano 1958) pointed to opposite conformation. Later work (Goto and Fieser, 1959) supported the conclusion based on rotatory dispersion data By analogy Klyne has suggested that *cis*-10-methyl-2-decalone probably exists in non-steriod form (Klyne, 1956) Such analogies are however not always unambiguous as is provided by the case of *cis*-10-methyl-2-decalone (Djerassi and Marshall 1958)

In the case of α β-unsaturated bicyclic ketones it appears that tricyclic ketone type of analogies are possible Thus the rotatory dispersion curves of (5S 10S) or (5S 10R)-5-hydroxy-10-methyl-$\Delta^{1(9)}$ 2-octalone (Djerassi, Osiecki and Herz 1957 Prelog and Acklin 1956) are found to be very similar to Δ^{4}-3-keto-steriods the similarity of the rotatory dispersion curve of carissone with Δ^{4}-3-keto-steriods points to β-orientation of the angular methyl group in this compound and α-orientation of the angular methyl group in the case of 1 14-dimethyl-2-keto-$\Delta^{1(11)}$-decahydro phenanthrene (Djerassi, Riniker and Riniker 1956b) The differences in the multiple Cotton effect curves of α-cyperone and epi-α-cyperone (Djerassi, Riniker and Riniker 1956) and, α- and β-vetivone (Djerassi Riniker and Riniker 1956b) are quite marked Djerassi, Riniker and Riniker (1956b) point to the similarities of the rotatory dispersion curves of helenalin and tenulin as providing additional evidence for the proposed structure of tenulin (Buchi and Rosenthal 1956)

Triterpenoids —The structure of triterpenoids and steriods is very similar In both cases the ring union A/B is *trans* (Barton and Holness 1952) and the angular methyl group occupies a similar position Rotational evidence showed clearly that they are all parallel enantiomeric type (Klyne 1952) This is further supported by asymmetric syntheses carried out by Prelog *et al* (1) and the synthesis of lanostenol from cholesterol (Barton, Ives Kelly Woodward and Patchett 1954b)

Barton and Jones (1944) were the first to correlate the rotation and structure in the three main groups of triterpenoids namely α-amyrin, β

amyrin and lupeol–betulin. They found that in these terpenoids Δ–value for acetylation benzoylation and oxidation at C–3 are very characteristic The ring unions of the β–amyrin group of triterpenoids are *trans–anti trans–anti–trans–syn–cis* (Barton and Holness 1952 Abd El Rehim and Carlisle 1954) and if the formulae of these compounds are rewritten following steriod conventions as suggested by Halsall, Jones and Meakins (1952) we find that the rings BCD are of the same type as the rings BCD of the A/B *trans* series steroids Klyne (1952) has tabulated rotational differences for intermediate rings in the β–amyrin terpenoids and the corresponding steroids It was found that molecular differences (Δ–values) for many (not all) olefinic, ketonic and conjugated groups in the two series are of similar sign and frequently of the same magnitude. The rotational differences of olefinic linkages in ring D and E generally support the structures based on chemical evidence (David 1949 Davy Halsall and Jones 1951 Davy Halsall Jones and Meakins 1951 Barton, Brooks and Holness 1951 b Barton and Holness, 1952) The Rule of Shift has also been applied to the nucleus and the side chain of lanosterol (Barnes Barton, Fawcett and Thomas, 1953 1954) In such studies of the Rule of Shift at present no satisfactory explanation is available in several cases of exceptions known.

The Cotton effect curves are often found to be very sensitive to conformational alterations and so rotatory dispersion studies were extended to this group of compounds (Djerassi Halpern Halpern and Riniker 1958 Djerassi Osiecki and Closson 1959) It is found that similar rotatory dispersion curves are given by compounds having the same conformation of rings and vice-versa. Thus dihydrobutyrospermone and masticadienonic acid have Cotton effect curves which resemble closely that of Δ –lanosten 3–one pointing to 9–α–orientation in dihydrobutyrospermone. Δ –cholesten 3β–ol–7–one acetate is found to exhibit multiple Cotton effect curve of enantiomeric sign to that of Δ^4–3–keto steroids and when two methyl groups are introduced in position 4 the rotatory dispersion curve becomes inverted recalling the pair testosterone and 6β– methyl testosterone. The rotatory dispersion curves of β–amyrone and Δ –lanosten–3–one resemble one another pointing to a common conformational denominator and thus providing a support to the earlier studies of Barton Head and May (1957) showing virtually identical condensation rates of these compounds with benzaldehyde. The rotatory dispersion curves of bauerenone and *iso*–bauerenone (Lahey and Leeding 1958) bear the same relationship to each other as those of Δ –lanosten 3–one and Δ^8–lanosten–3–one pointing to a parallel conformational relationship The similarity of the rotatory dispersion curves of gypsogenin methyl ester and quillaic acid methyl ester indicate identical stereochemical attachment of the aldehyde function. The rotatory dispersion can indicate the stereochemical assignment of methyl groups adjacent to the carbonyl group. In the case of α β–unsaturated triterpenoid ketones rotatory dispersion studies appear to help in indicating stereochemical and conformational alterations. At

present, however, the available data is meagre and further study alone can show the extent of reliability of such rotatory dispersion studies in solving stereochemical and conformational problems in triterpenoids.

Diterpenoids are closely related to triterpenoids. The work dealing with the chemical correlation of the A/B ring in these types of compounds has been reviewed by Barton (1949) and by Ruzicka (1953). Barton suggested that abietic acid has substituents at C–13 and C–12 *trans* which makes it an analog of cholesta-3,5-diene and it is found that the Δ-values for the conjugated double bonds in these compounds are of the same sign and order of the magnitude. In a like manner optical rotation points that in laevopimaric acid C_{13}–H is *cis* to C_{13}–CH_3. Neoabietic acid (Harris and Sanderson 1948 a, b) on the basis of optical rotation is apparently analogous to 5-methyl-enecholest-4-ene (Musgrave 1951). Optical rotation has also been used (Vogel, Jeger and Ruzicka 1951) to correlate the stereochemistry of the carboxyl groups in gypsogenin and α-boswellic acids (triterpenoids) and abietic acid and pedocarpic acid (diterpenoids).

In the case of other terpenoids the optical rotation studies have been mostly confined to menthol and camphor derivatives. Isomeric menthols, menthylamines and related compounds were studied by Read (1930; Read and Grub 1934 a, b). Rule (1930) found the $(M)_D$ values for nine substituted acetates of (−)-menthol to range from −157° to −174° as compared with −157° for un-substituted ester indicating that the rotation of the ester is more or less independent of the nature of the symmetrical component. Rupe (1914; Rupe *et al* 1930, 1932, 1940) studied the validity of the principle of superposition, and, unsaturation in camphor derivatives. He examined the rotatory power of esters obtained from optically active alcohols and optically active acids (both camphor derivatives). It was noted that the contributions were more or less additive if both the components of the ester were saturated or even if one of them was unsaturated. However serious discrepancies occurred if both the components were unsaturated. The work of Rupe *et al* has been reviewed in the obituary of H. Rupe by Dahn and Reichstein (1952). The sensitivity of optical rotation even at points away from asymmetric centre is amply illustrated by the study of amino derivatives of oxymethylene camphor and ( )-camphor-10-sulphonic acid. In these derivatives the rotatory dispersion in the visible region is simple and can be expressed by Drude's one term equation. In table 5 is given the specific rotatory power of anilino derivatives of oxymethylene camphor in different solvents (Singh, Bhaduri and Barat 1931; Singh and Bhaduri 1937, 1939, 1947; Singh and Barat 1940; Singh and Lal 1940; Singh and Sen 1943; Singh and Tewari 1945, 194[illegible]

TABLE 5

Rotatory power of arylaminomethylene camphors in different solvents

No.	$R=C_8H_{14}<\genfrac{}{}{0pt}{}{C=CH-}{CO}$	Me.OH	EtOH	C_5H_5N	$CHCl_3$	Acetone	Benzene
		$[\alpha]^{35}_{5461}$ in solvent					
1	R.NH-C_6H_5	480 0	451 5	433 2	4.4 6	448 9	367 1
2	R NH-C_6H_4-Cl	400 2	405 8	391 5	386 0	380 0	364 7
3	R NH-C_6H_4-Br	347 5	339 9	328 4	329 0	330 1	314 3
4	R.NH-C_6H_4-NO_2	493 1		421 8	481 6	408 1	399 2
5	R NH-C_6H_4-COOH	388 5	388 5	377 3	382 2	373 5	395 6
6	R.NH-C_6H_4-Cl	388 5	381 7	374 4	381 4	390 0	361 8
7	R.NH-C_6H_4-Br		334 0	331 5	321 1	348 4	279 3
8	R NH-C_6H_4-NO_2	393 5		367 1	350 0	389 2	315 0
9	R NH-C_6H_4-COOH	396 8	390 9	371 5		370 1	
10	R.NH-C_6H_4-CH_3	451 5	439 9	405 8	384 8	442 3	332 6

(Continued on the next page)

TABLE 3—(Contd)

Rotatory power of arylaminomethylene camphors in different solvents

S. No.	$R-C_8H_{14}<$ (C=CH–, CO)	(α) 35/5161 in solvent: MeOH	EtOH	C_5H_5N	$CHCl_3$	Acetone	Benzene
11	R NH–C_6H_4–I (ortho)	328 5	319 5	291 8	279 1	309 7	251 1
1	R NH–C_6H_4–Cl	417 7	408 3	391 4	374 6	401 8	350 4
13	R NH–C_6H_4–Br	361 1	355 6	348 8	324 3	360 6	291 9
14	R NH–C_6H_4–I	338 1	325 9	318 4	300 0	326 5	258 6
15	R NH–C_6H_4–NO_2	510 7	..	545 0	493 5	519 4	437 0
16	R NH–C_6H_4–COOH	..	..	426 3	...	..	..
17	R NH–C_6H_4–CH_3	463 0	440 8	401 6	401 6	425 8	333 4
18	R NH–C_6H_4–NH_2	484 4	475 1	..	428 8	461 8	..
19	R NH–C_6H_4–$N(CH_3)_2$	..	455 9	420 1	470·0	450 9	...
2	R NH–C_6H_3(Cl)–Cl	360 0	351 0	358 0	356 0	352 0	344 0

(Continued on the next page)

TABLE 5—(Contd.)

Rotatory power of arylaminomethylene camphors in different solvents

S. No.	$R = C_8H_{14}\langle^{C=CH-}_{CO}$	$(\alpha)^{35}_{5461}$ in solvent MeOH	EtOH	C_5H_5N	$CHCl_3$	Acetone	Benzene
21	R.NH–⬡ (NO₂, Cl)	380 0	365 0	350 0	320 0	360 9	290 0
22	R.NH–⬡ (NO₂, CH₃)	388 0	376·0	362 0	330 0	368 0	295·0
23	R.NH–⬡ (NO₂, CH₃)	350 0	352 0	370 0	355 0	355 0	339 0
24	R.NH–⬡ (Cl, CH₃)	388 0	379 0	390 0	373 0	380 0	361 0
25	R.NH–⬡ (NO₂)–CH₃	492 9	459 5	416 4	458 3	461 1	390 0
26	R.NH–⬡ (CH₃)–NO₂	491 8	500 7	501 5	523 3	522 1	479 4
27	R–N=CH–⬡ (NO₂)	319 7	301 9	261 5	301 7	305 6	—
28	R–N=CH–⬡ (NO₂)	323 0	313 3	281 0	300 8	303 9	—
29	R–N=CH–⬡–NO₂	335 0	326 2	232 0	321 9	318 1	
30	R–N=CH–⬡–N(CH₃)₂	188 2	197 8		107 2	172 4	—

Extrapolated values

In table 6 is given the rotatory power of amino derivatives of (+)-camphor 10-sulphonic acid in non-aqueous solvents (Singh Perti and Singh 1944; Singh and Perti 1945 a, b; Singh and Manhas 1947 1948 1949 Singh Mital and Manhas, 1950-51 Singh and Kapoor 1951 1952; Perti and Rastogi 1955 1956 Perti and Agnihotri 1958a, b Perti and Pant, 1959a, b 1960 Pant, 1960)

TABLE 6

Rotatory power of amino derivatives of (+)-camphor-10-sulphonic acid in non-aqueous solvents

S. No.	R = CH_2—C(CH_2—H—)—CO, CH_3—C—CH_3, CH_2—CH—CH_2	$(\alpha)_{5461}$ in solvent					
		MeOH	EtOH	C_6H_6	AcOEt	$CHCl_3$	Acetone
1	R SO_3 H_2N–⬡	32 40	36 40	36 10	—	—	–
	R SO_3 H_2N–⬡ (NO_2)	29 97	29 90	31 96	—	—	28 98
3	R SO_3 H_2N–⬡ (COOH)	30 50	32 50	36 00	—	–	–
4	R. SO_3 H_2N–⬡ (CH_3)	29 60	34 74	36 20		–	—
5	R SO_3 H_2N–⬡	28 50	32 00	36 00	—	—	—
6	R SO_3 H_2N–⬡ (Cl)	6 96	28 97	32 50	—	—	—
7	R SO_3 H_2N–⬡ (Br)	35 00	35 00	40 00	36 00	32 00	–
8	R SO_3 H_2N–⬡ (OCH_3)	–	31 39	30 82	—	–	—
9	R SO_3 H_2N–⬡ (OH)	28 00	—	–	—	66 00	—
10	R SO_3 H_2N–⬡ (NO_2)	34 47	35 36	35 97	—	–	30 47

(Continued on the next page)

TABLE 6—(Contd.)

Rotatory power of amine derivatives of (+)-camphor-10-sulphonic acid in non-aqueous solvents

S. No.	R = CH_2—C(CH_2–H–)—CO; CH_3–C–CH_3; CH_2—CH—CH_2	(α) 5461 in solvent					
		Me OH	Et.OH	C_5H_5N	Ac.OEt	$CHCl_3$	Acetone
11	$R\ SO_3\ H_2N-C_6H_4-COOH$	29·00	30 00	31 50		...	...
12	$R\ SO_3\ H_2N-C_6H_4-CH_3$	28 90	34 10	35 50	...		...
13	$R\ SO_3\ H_2N-C_6H_4-I$	28 00	33 00	34 50	...	..	...
14	$R\ SO_3\ H_2N-C_6H_4-Cl$	32 00	24 00	34 46	...	...	..
15	$R.SO_3\ H_2N-C_6H_4-Br$	36·00	34 00	57 00	57 00	45 00	
16	$R.SO_3\ H_2N-C_6H_4-OH$	40 00	30 00	...		45 00	
17	$R.SO_3H_2N-C_6H_4-NO_2$	30 47	30 42	31 42	...		28 45
18	$R.SO_3H_2N-C_6H_4-COOH$	27 00	31 00	33 50			..
19	$R\ SO_3\ H_2N-C_6H_4-CH_3$	30 47	33 50	37 00	..	...	
20	$R.SO_3\ H_2N-C_6H_4-I$	29 50	33 00	33 00			

(Continued on the next page)

TABLE 6—(*Contd*)

Rotatory power of amine derivatives of (+)-camphor-10-sulphonic acid in non-aqueous solvents

R = camphor-10-sulphonyl skeleton:

```
            CH2-R-
             |
CH2 -------- C -------- CO
 |           |           |
 |       CH3-C-CH3       |
 |           |           |
CH2 -------- CH -------- CH2
```

S No.	Compound	(α) 5461 in solvent: MeOH	EtOH	C_5H_5N	AcOEt	$CHCl_3$	Acetone
1	R SO_3 H_2N-C_6H_4-Cl	31 50	33 .0	36 00	--		
22	R SO_3 H_2N-C_6H_4-Br	38 00	32 00	40 00	30·00	43 00	..
23	R. SO_3 H_2N-C_6H_4-OCH_3	..	31 59	33 61	...	..	..
24	R SO_3 H_2N-C_6H_4-CO-CH_3	..	31 61	...	...	33 75	..
25	R. SO_3 H_2N-C_6H_4-OC_2H_5		40 90	36 05	...	...	..
6	R $SO_3$$H_2N$-$C_6H_4$-OH	31 00	..	..	...	30 00	..
27	R SO_3 H_2N-C_6H_4-SO_2 NH_2	28 00	...	33 47	...	...	
28	R. SO_3 H_2N-C_6H_4-N N-C_6H_5	...	...	15 00	..	...	...
29	R SO_3 H_2N-C_6H_4-SO_2-NH-C_5H_4N	96 18	108 2	...	...	...	120·2
30	R SO_3 H_3N	43 51	48 94	...	...	...	..

(α) 6708

(Continued on the next page)

TABLE 6—(Contd.)

Rotatory power of amine derivatives of (+)-camphor 10-sulphonic acid in non-aqueous solvents

S. No.	R = CH_2–H– ; CH_2—C—CO ; CH_3—C—CH_3 ; CH_2—CH—CH_2	$(\alpha)_{5461}$ in solvent					
		MeOH	EtOH	C_5H_5N	AcOEt	$CHCl_3$	Acetone
31	$R.SO_3\ H_2N\ CH_3$	43.25	45.00	37.50	...	41.08	...
32	$R.SO_3\ H_2N\ CH_2\ CH_3$	40.82	43.29	..	...	...	
33	$R.SO_3\ H_2N\ CH_2\ CH_2\ CH_2\ CH_2\ CH_3$	36.67	38.50	45.50	...	46.00	
34	$R\ SO_3\ H_2N\ CH_2\ CH_2\ OH$	38.03	40.11	41.88		34.69	...
35	$R.SO_3\ H_2N\ CH_2\ CH = CH_2$	36.27	37.49	46.83	...	39.59	...
36	$R\ SO_3\ H_2N\ CH(CH_3)_2$	41.49	43.05	39.80		46.00	...
37	$R.SO_3\ H_2N$–C_6H_2(Br)(Br)(Br)		23.00	22.30	..	21.00	...
38	$R.SO_3\ H_2N$–C_6H_2(Br)(Cl)(Cl)	27.45	29.93	30.00		...	...
39	$R.SO_3\ H_2N$–C_6H_2(CH_3)(Cl)(CH_3)	38.00	39.00			50.00	...
40	$R.SO_3\ H_2N$–C_6H_2(CH_3)(CH_3)(Cl)	42.00	40.00			52.00	...

(Continued on the next page)

TABLE 6—(*Contd.*)

Rotatory power of amino derivatives of (+)-camphor-10-sulphonic acid in non-aqueous solvents

R = camphor-10-sulphonyl skeleton: CH_2—C(—CH_2—H—)—CO; CH_3—C—CH_3; CH_2—CH—CH_2

S. No.		$(\alpha)_{5461}$ In solvent MeOH	EtOH	C_5H_5N	AcOEt	$CHCl_3$	Acetone
41	$R.SO_3\ H_2N$– (CH_3, CH_3)	39 00	42 50	..	..	50 00	
42	$R\ SO_3\ H_2N$– (CH_3, CH_3)	42 00	40 50	...	...	45 00	..
43	$R\ SO_3\ H_2N$–	28 00	30 00	33 97	...	..	
44	$R\ SO_3\ H_2N$=	31 42	31 50	33 98	...	...	...
45	$R\ SO_3\ H_2N$–	21 97	36 47	39 00	...	...	...
46	$R\ SO_3\ H_2N$– (N)	94 51	109 1	107 6	...	130 4	...
47	$R\ SO_3\ H_2N$–C (N—CH, CH, S)	101 3	119·5	108 8	...	148 1	–
48.	$R\ SO_3\ H_2N$– (OCH_3)	91 35	115 7	103 0	...	161 1	125 9

In these derivatives (tables 5 6) the rotation differences are small indicat ing that the main contribution to optical rotatory power is not much effected by constitutional changes at a point remote from active centre. However there are slight differences in rotatory power and it is generally found that the substitution of an electropositive group in place of hydrogen causes a slight enhancement in rotation and an opposite effects is seen if hydrogen is replaced by an electronegative group The magnitude of rotatory power is found to be slightly different in different solvents indicating the importance of choice of solvent in all comparisons of rotatory power It is also worth noting that even in these derivatives introduction of unsaturation causes a marked change in rotatory power

Arylamino derivatives of camphor obtained by condensation of arylamines with camphorquinone (Lowry and Cutter 1925) have also been extensively studied by Singh *et al.* Singh and Mazumdar (1919) studied the rotatory power of some naphthyl mino camphors and derivatives of phenylimino camphor obtained by condensat on of camphorquinone with o–,m– and p–toluidine, o–,m– and p–bromanilines o– and m–chloranilines, α– and β–naphth ylamine, aniline, o– and m–phenylene diamine and o–anisidine. This work was later extended to m–toluene*bis*iminocamphor m–toluene*bis*aminocamphor and ar–tetrahydro–α–naphthyliminocamphor (Singh, Singh Dutt and Singh, 1920) A high molecular rotatory power is displayed by products of condensation of camphorquinone with arylamines (Forster and Thornley 1909) Singh and Singh (1920) noted the remarkably high rotatory power of 1 4–naphthalene-*bis*-iminocamphor [$(M)_D$=8175 in chloroform] Singh Singh and Lal (1921) found high rotatory power in o–o –ditolyl*bis*iminocamphor o–o –dimethoxydiphenylene*bis*iminocamphor p–p –d phenylene*bis*iminocamphor and p–p′–*bis*iminocamphor diphenylamine, and noted that the effect of the substituents in order of diminishing rotatory power is H > CH_3 > OCH_3. The measurement of absorption spectra (Singh and Rai 1926) of these types of compounds shows that the compounds wh ch possess a greater number of unsaturated conjugated linkages have greater absorption as well as greater rotation The reduction of the azethenoid group (Singh and Bhaduri, 1930[a]) in p–phenylene*bis*iminocamphor p–p –diphenylmethane*bis*iminocamphor and p–iminocamphor diphenylamine causes a phenomenal lowering in the rotatory power The effect is much less marked if the conjugation in the side chain (i outside the terpene nucleus) is broken (Singh and Bhaduri, 1931[b]) That unsaturation does not always lead to enhancement of rotation in such derivatives was observed by Forster and Seville (1921) Singh and Bhaduri (1931[b]) found a similar irregularity in the case of lower rotation of o–o –stilbene*bis*iminocampho as compared to o–o –dibenzyl*bis*imino camphor Generally however unsaturation is found to cause an increase in rotatory power as can be seen by a comparison of rotatory power of aryl derivatives of aminomethylene camphor which form an intermediate series of compounds to the corresponding derivatives of iminomethylene camphor and aminomethy lene camphor (Singh Bhaduri and Barat, 1931 Singh and Bhaduri 1931)

The unsaturated groups if present in conjugated form are more effective (Singh and Bhaduri 1932) and if conjugation is destroyed there is a phenomenal fall in the magnitude of rotatory power (Singh and Kapoor 1949) In these compounds the quinoline ring appears to have a lower rotatory contribution than the naphthalene ring (Singh and Kapoor 1950). In the case of phenyl o- m- and p-tolylamino and imino camphors, the rotatory power of amino derivatives is only about 10 % of that of the corresponding imino derivatives (Singh and Singh, 1951) The rotatory dispersion of o-,m- p-chlorophenylimino-(+)-camphors and phenyl, o-,m- and p-chlorophenylamino-(+)-camphors were studied by Singh and Seth (1956) who found that the remarkable fall in the molecular rotatory powers of arylimino camphors on their reduction to arylamino compounds may be correlated with a break in the conjugation of the double bonds of the carbonyl, azethenoid and phenyl groups present in the arylimino derivatives. It was also noticed that the fall in rotation is most in the *para* substituted compounds and least in the *ortho* substituted ones The fall in the unsubstituted compounds was found to be intermediate between those of *para* and *meta* derivatives Similar results were obtained by Singh and Saxena (1958) in their study of rotatory dispersion of o- -m -p-methoxy and ethoxy phenylimino-(+)-camphor Most of the camphorquinone derivatives described above show simple rotatory dispersion and their dispersion data can be expressed by one term Drude equation. In the case of p-iodophenylamino camphor however the rotation in chloroform (4) can be expressed by simple Biot's equation (Singh Basu-Mullick and Bhaduri 1931)

(+)-Camphor-β-sulphonyl arylamides show simple dispersion and it is found that a change of substituent in the aryl part of the molecule has influence on the magnitude of the rotatory power Singh and Amma (1954) studied the rotatory dispersion of (+)-camphor-β-sulphonyl-phenyl-and o-m- and p-chlorophenylamides This work was later extended to the determination of rotatory dispersion of (+)-camphor-β-sulphonyl (o-,m p-)-bromophenylamides (Singh and Amma 1956) It was found that the rotation is generally controlled by a low frequency corresponding to the ketonic group of camphor and sometimes by a high frequency characteristic of the saturated molecule Singh Verma and Murty (1956) examined the rotatory dispersion of (+)-camphor-β-sulphonyl-phenyl o-tolyl, m-tolyl p-tolyl α-naphthyl and β naphthylamide It was found that (+)-camphor-β-sulphonyl phenyl amide exists in dimorphic forms (α- and β) and both the forms have identical rotatory dispersion and, the transformation of α- into β- form takes place at about 118 C Singh and Verma (1957 & 1958) later extended this work to the determination of rotatory dispersion of (+)-camphor-β-sulphonyl-nitrophenylamides (o m- and p-) (+)-camphor β-sulphonyl-methoxyphenylamides (o- m- and p-) (+)-camphor β-sulphonyl-ethoxy phenylamides (o- m- and p-) (o m- and p-)-phenylene-*bis* (+)-camphor β-sulphonylamides and, (+)-camphor β sulphonyl derivatives of benzidine tolidine dianisidine, 4 4'-diamino-diphenyl methane and 4 4'-diamino-diphenyl sulphone All

these derivatives showed simple dispersion and their rotatory power in the visible region of the spectrum could be expressed by Drude's one term equation. Here it may be pointed out that (+)-camphor-β-sulphonamide shows anomalous rotatory dispersion (Richards and Lowry 1925) in 10% solution in chloroform but its rotatory dispersion in methanol, pyridine, acetone, ethyl acetate or benzene is simple (Singh and Amma, 1956) It is interesting to note that while D-camphor (Lowry and Cutter 1925) and D-camphor-β-sulphonamide are *dextro* rotatory D-camphor-β-sulphonanhydramide is *laevo* rotatory Though the preparation of these compounds was described by Reychler it was the work of Lowry and Armstrong (1902) which showed that these compounds were not isomers but the one having higher melting-point was the anhydride of the other The kinetics of conversion of D-camphor-β-sulphonamide into D-camphor-β-sulphonanhydramide in methanol or acetone in presence of sodium ethoxide showed that the reaction was monomolecular (Singh and Amma, 1956)

Amongst other terpenoid derivatives studied mention be made of the following camphoramide (Tefel and Eckstein 1901) camphoric anhydride (Lowry 1903) menthyl and bornyl xanthate and, menthyl bornyl and fenchyl dithiourethanes (Tschugaeff 1909 Tschugaeff and Ogorodnikoff 1910 1911 1912, 1913 Lowry and Hudson, 1933); camphorimide, camphor benzylamide, benzyl camphoramic acid (Singh and Biswas 1924) α-bromo-camphor and αα-dibromo camphor and the corresponding chloro compounds (Cutter Burgess and Lowry 1925) Camphor-β-sulphonic acid (Richard and Lowry 1925) phenyl o- m- and p-tolyl and β-naphthyl derivatives of (+)-camphorimide and (+)-camphoramic acid (Singh and Puri, 1926) camphor carboxylic acids, camphors and borneols (Singh and Nayar 1948) santonine group of sesquiterpenoids (Huang-Minlon 1948 Barton 1950) longifolene and camphene (Ourisson, 1953) phenyl o-nitrophenyl p-nitrophenyl and 2 4-dinitrophenyl hydrazones of (+)-camphor β-sulphonic acid (Manhas and Banerji 1956)

Inspite of the large amount of rotatory power data available in the case of camphor derivatives and related compounds, no systematization of structural relationships has yet been made. The structural and conformational problems in camphor and related compounds are yet to be systematically analysed The data available, however emphasizes that the effect of structural factors in one series of compounds is not necessarily the same in another series of compounds howsoever closely related Great caution is, therefore, needed in extending analogies based on optical rotation data alone

6 *Others*—Application of rotational data to structural correlation has been attempted in other types of compounds also These attempts have, however not always given unambiguous results. The most important of these groups of compounds are alkaloids Embde (1930) attempted the application of rotational evidence to structural correlations among morphine group of alkaloids. This attempt was criticised by Freudenberg and Kuhn

(1931). A similar attempt has been made in the quinine group of alkaloids (Embde, 1932; Prelog and Hafliger, 1950). Leithe (1930a, b, 1931, 1934) applied rotational evidence to structural problems in laudanosine series. A similar correlation has been tried in the yohimbine group of alkaloids (Klyne, 1953).

Some alkaloid molecules appear to be quite sensitive to changes in optical rotatory power. Minguin (1905) prepared some salts of strychnine with organic acids and investigated their optical properties in a mixture of benzyl and ethyl alcohols. Hilditch (1908) used alkaloids like strychnine, brucine, quinine etc. for preparing optically active salts. Salts of brucine with benzoic and substituted benzoic acids have been studied by Manhas and Banerji (1957 b, 1959a; Banerji 1957) who found that these compounds exhibited simple rotatory dispersion which could be expressed by Drude's one term equation. Various substituents in the benzene part of these compounds were found to have an effect on the optical rotatory power. In the case of *ortho* substitution the order of rotatory power was $OH < NH_2 < I < H < Br < Cl < NO_2$; *meta* substitution gave the order $OH < H < I < Br < NO_2 < Cl$, and *para* substitution $OH < I < Br < NO_2 < NH_2 < Cl$. A similar study of strychnine salts of benzoic and substituted benzoic acids (Pertl, Pant and Ghildyal, 1960; Pant, 1960) indicated that substituents in the benzene part of the molecule effect the magnitude of optical rotatory power. The effect of nitro group is specially very marked and causes an abrupt decrease in rotation (Pertl and Pant, 1960). Benzoic and substituted benzoic acid salts of quinine (Manhas and Banerji, 1959b) exhibit similar sensitiveness of optical rotatory power to substituents in the aromatic part of the molecule. Salts of acetic, propionic, butyric, phenyl acetic, β-phenyl propionic and cinnamic acid with cinchonidine also show similar results (Manhas and Shukla, 1960). These studies point to the danger of drawing conclusions regarding structural or conformational make-up of a molecule simply on the basis of slight rotational differences.

Djerassi and Krakower (1959) have measured rotatory dispersion of a series of 3-methyl cycloalkanones with a view to trace the effect of the ring size in these compounds. Cyclisation is known generally to cause an enhancement in rotation (Kauzmann, Walter and Eyring, 1940; Mislow and Hamermesh, 1955). The rotatory dispersion studies of Djerassi and Krakower indicate that in six or seven membered ketones the conformation is other than the chair form. Djerassi and Geller (1959) have measured the rotatory dispersion of a series of homologous aldehydes (Levene and Rothen, 1936) and methyl ketones. These results have been used as the basis of analogy to suggest absolute configuration of phytol (Crabbe, Djerassi, Eisenbraun and Liu, 1959).

A comparative study has been carried out between ultraviolet absorption spectra of steroidal six membered ketones and their hydroxy and acetoxy derivatives (Cookson and Dandegaonker, 1955; Baumgartner and Tamm, 1955; Lek, Phillips, Walker and Wyman, 1956). It was noticed that

an *axial* hydroxy group produces a *bathochromic* shift of 14–20mμ and the axial acetoxy of about 10mμ, while an *equatorial* substituent results in a *hypsochromic* change of 9 to 13mμ in the case of hydroxy and about 5mμ in the case of acetoxy Such shifts could also be qualitatively observed in the Cotton effect curves of some appropriate ketols (Djerassi, Halpern Halpern Schindler and Tamm, 1958) Similar absorption spectra of α-haloketones (Cookson 1954 Cookson and Dandegaonker 1955) and their rotatory dispersion measurements (Djerassi Osiecki, Riniker and Riniker 1958 Djerassi Fornaguera and Mancera, 1959) in the steriod field showed that the introduction of *equatorial* halogen in either adjacent position of a keto group in a cyclohexanone does not alter the sign of the Cotton effect of the halogen free ketone but an *axial* chlorine or bromine atom next to the keto group of a cyclohexanone may effect the sign of the Cotton effect curve of the parent ketone (Djerassi and Klyne, 1957) Recently Jones and Wałuka (1959) have confirmed the *axial* nature of a bromine atom in the case of 8β-bromo-cholesten-3β-ol-7-one acetate based on these considerations The available results in such studies are at present meagre and such analogies have to be used very carefully

Since 1956 rotatory dispersion measurements are being increasingly used for the determination of absolute configuration. The method used, in principle is similar to the generalised method of monochromatic (usually D-line) molecular rotation differences (Klyne 1952 1953) Comparison of rotatory dispersion curves is generally more suitable as it involves comparisons for many wavelengths It may be mentioned that rotation at D-line is frequently a reflection of the sign of dispersion curve in the ultraviolet (Kuhn and Biller 1935a) except when the dispersion curve changes sign somewhere between the visible and the ultraviolet. In certain cases such as longifolene (Jacob Ourisson and Rassat 1959) the comparison of rotatory dispersion curves simply confirmed the conclusions already reached by a consideration of rotation data for one wavelength In other cases such as the guaianolide group of sesquiterpenes (Djerassi, Osiecki and Herz 1957 Dolejs, Soucek, Horak Herout and Sorm 1958) the comparison of rotatory dispersion curves has afforded the only information available at present. In certain cases such as those of cafestol (Djerassi, Cais and Mitscher (1958 1959) iresin (Djerassi and Burstein 1958) and gibberellic acid (Stork and Newman, 1959) it provided very convincing evidence while in others such malliol (Buchi, Wittenau and White, 1959 Baumann and Prelog 1958) it can only be considered as tentative because of possible conformational complications. The subject has been recently reviewed by Djerassi (1959)

The direct application of optical rotation to structural problems is based on analogies. These analogies to be useful must be sound During the last five years, particularly after the introduction of a photoelectric spectropolarimeter (Brand Washburn Erlanger Ellenbogen, Daniel Lippmann and Scheu 1954 Rudolph 1955) enabling rapid measurements of rotatory dispersion over an appreciable ultraviolet spectral range, there has been a

sudden burst of activity in this field As more and more measurements are being made it is being increasingly realised that the rotatory dispersion curve is quite sensitive to constitutional, stereochemical and conformational changes. Anologies are being extended as soon as sufficient data regarding reference compounds is gathered There are still many fields open for investigation where little or no work has been done on the correlation of rotations or rotatory dispersions. There is little doubt that measurement of rotatory dispersion is going to play an increasingly vital role in the correlation of constitutional and conformational studies in many types of organic compounds

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REPRESENTATION THEOREMS FOR A GENERALIZED STIELTJES TRANSFORM

SURESH CHANDRA ARYA
Professor of Mathematics (Indian Aid Mission)
Tribhuvan University Kathmandu Nepal.

INTRODUCTION

If we iterate the Laplace transform we get the Stieltjes transform i.e., if we take

(1.1) $f(s) = \int_0^\infty e^{-st} \phi(t)\, dt$

where

(1.2) $\phi(s) = \int_0^\infty e^{-st} \phi(t)\, dt$

then

(1.3) $f(s) = \int_0^\infty (s+t)^{-1} \phi(t)\, dt$

and the transform (1.3) is referred to as the Stieltjes transform.

Varma (1951) has given a generalization of the Laplace transform in the form

(1.4) $f(s) = \int_0^\infty (st)^{m-\frac{1}{2}} \quad e^{-\frac{1}{2}st} \quad W_{k,m}(t) \quad \phi(t)\, dt$

$W_{k,m}$ denotes the Whittaker function Further he has shown that if we take $f(s)$ to be the transform of $\phi(t)$ in the sense of (1.4) and $\phi(\ )$ to be the ordinary Laplace transform of $\phi(t)$ in the sense of (1.2) then we have formally

(1.5) $f(s) = \frac{\Gamma(2m+1)}{s\Gamma(m-k+\frac{3}{2})} \int_0^\infty F(2m+1 \quad 1 \quad m-k+\tfrac{3}{2} \quad -t/\ )\ \phi(t)\, dt$

If $k+m=\frac{1}{2}$ the hypergeometric function degenerates into a binomial expression and (1.5) reduces to (1.3) Also if $k-m=\frac{1}{2}$ and $2m+1=\rho$ (1.5) reduces to the transform

(1.6) $\phi(s) = \Gamma^{1-}(\rho) \quad ^{-\rho+1} \quad f(\ ) = \int_0^\infty (\ +t)^{-\rho} \ \phi(t)\, dt$

In this paper we give representation theorems for the generalized Stieltjes transform (1.5) We also give a representation theorem for the Stieltjes integral

(1.7) $f(s) = \frac{\Gamma(2m+1)}{s\Gamma(m-k+\frac{3}{2})} \int_0^\infty F(2m+1 \quad 1 \quad m-k+\tfrac{3}{2}; \ -t/\ )\ d\ \alpha(t)$

where $\alpha(t)$ is a function of bounded variation in (R) for every positive R We assume that s is real and positive.

REPRESENTATION THEOREMS FOR GENERALIZED STIELTJES-LEBESGUE INTEGRAL

In this section we give representation theorems for the generalized Stieltjes-Lebesgue integral (1.5)

Theorem 2.1 If $f(s)$ has the derivatives and integrals of all orders in $0<s<\infty$ which satisfy the conditions

$$(2.1)\quad f^{(n)}(s)=o(s^{-n-1})\ (s\to 0+,\ n=0,1,2,\ldots)$$
$$=o(s^{-n})\ (s\to\infty,\ n=0,1,2,\ldots)$$

then

$$\lim_{n\to\infty}\frac{\Gamma(2m+1)t^{-1}}{\Gamma(m-k+3/2)}\int_{0+}^{\infty}F(2m+1,1;m-k+3/2;-s/t)\,L_{n,s}\,f(t)\,ds$$
$$=f(t)\qquad(0<t<\infty)$$

provided that $Re(2m+1)>0$, $m-k+3/2\neq 0,-1,-2,\ldots$ where (Arya 1964)

$$L_{n,s}f(t)=\frac{\Gamma(2n+m-k+\tfrac{1}{2})\,\Gamma(2n+m+k-\tfrac{1}{2})\,(-1)^{n-1}}{\Gamma(2m+2n)\,\Gamma(n+1)\,\Gamma(2n)\,\Gamma(n+m+k-3/2)}\ P_{n,s}\,f(t)$$

and

$$P_{n,s}\,f(t)=D^{n}\,s^{2n-1}\,D^{n-1}\,s^{2m+n-1}\,D^{n-1}\,s^{-k-m+\frac{1}{2}}\,D^{1-n}\,s^{-m+k-n+\frac{1}{2}}f(s)$$

Proof. Let us set

$$(2.2)\quad g(s,t)=\frac{\Gamma(2m+1)\,t^{-1}}{\Gamma(m-k+3/2)}\,F(2m+1,1;m-k+3/2;-s/t)$$

Then

$$D^{n}g(s,t)=\frac{\Gamma(2m+1+n)\,\Gamma(n+1)}{\Gamma(m-k+3/2+n)}\,(-1)^{n}\,t^{-n-1}$$
$$F(2m+1+n,1+n;m-k+n+3/2;-s/t)\qquad D\equiv(d/ds)$$

Then for a fixed positive t we have (Erdelyi etc. 1953, p. 63)

$$(2.3a)\quad F(2m+1+n,1+n;m-k+n+3/2;-s/t)\sim$$
$$\frac{\Gamma(m-k+n+3/2)\,\Gamma(-2m)}{\Gamma(n+1)\,\Gamma(-m-k+\frac{1}{2})}(s/t)^{-2m-1-n}+\frac{\Gamma(m-k+n+3/2)\,\Gamma(2m)}{\Gamma(2m+1+n)\Gamma(m-k+\frac{1}{2})}$$
$$(s/t)^{-n-1}\ (s\to\infty)$$

if $2m$ is not an integer or zero

$$(2.3b)\quad \frac{\Gamma(2m+1+n)}{\Gamma(m-k+n+3/2)}F(2m+1+n,1+n;m-k+n+3/2;-s/t)\sim$$
$$\sim(-1)^{2m}\frac{\Gamma(2m+1+n)\,(s/t)^{-2m-1-n}}{\Gamma(-m-k+\frac{1}{2})\,(2m)!\,n!}\Big[\log(s/t)+\psi(2m+1)+\psi(1)-$$
$$-\psi(2m+1+n)-\psi(-m-k+\tfrac{1}{2})\Big]+\frac{\Gamma(2m)}{\Gamma(m-k+\frac{1}{2})}\,(s/t)^{-1-n}\qquad(s\to\infty)$$

if $2m$ is zero or a positive integer and $m-k+\frac{1}{2}\neq 0$ or a positive integer the last term being zero when $2m=0$

$$(2.3c)\quad \frac{\Gamma(2m+1+n)}{\Gamma(m-k+n+3/2)} F(2m+1+n,\ 1+n,\ m-k+n+3/2,\ -s/t)$$

$$\sim(-1)^{m-k+\frac{1}{2}}\ (s/t)^{-m+k-n-3/2}\ \frac{(1+n)_{m-k+\frac{1}{2}}}{(m-k+\frac{1}{2})!}$$

$$\frac{(-1)^{2m}(s/t)^{-2m-1-n}(1+n)_{2m}}{(2m)!\,(-m-k-\frac{1}{2})!}$$

$$[\log (s/t)+\psi(2m+1)+\psi(1)-\psi(2m+1+n)-\psi(-m-k+\tfrac{1}{2})]$$

$$+\frac{(2m-1)!}{(m-k-\frac{1}{2})!}\ (s/t)^{-n-1}\qquad (s\to\infty)$$

if $2m$ is zero or a positive integer and $-m-k+\frac{1}{2}$ is zero or a positive integer the second and third terms being zero when $-m-k+\frac{1}{2}=0$ or $2m=0$

The function $\psi(z)$ is the logarithmic derivative of the gamma function i.e.

$$\psi(z)=(d/dz)\log\Gamma(z)$$

Also

$$(2.4)\quad F(2m+1+n,\ 1+n,\ m-k+n+3/2,\ -s/t)=o(1)\qquad (s\to 0+,\ n=0,1,2,\ldots)$$

Let us set

$$I=\int_{0+}^{\infty} g(s,t)\ P_{n,s} f(t)\ ds$$

$$=\int_{0+}^{\infty} g(s,t)\ D^{n}\ s^{2n-1}\ D^{n-1}\ s^{2m+n-1}\ D^{n-1}\ s^{-k-m+\frac{1}{2}}\ D^{-n+1}\ s^{-m+k-n+\frac{1}{2}}\ f(s)\ ds$$

Then on integrating by parts we have

$$I=\Big[g(s,t)D^{n-1}\ s^{2n-1}\ D^{n-1}\ s^{2m+n-1}\ D^{n-1}\ s^{-k-m+\frac{1}{2}}\ D^{-n+1}\ s^{-m+k-n+\frac{1}{2}}\ f(s)\Big]_{s=0}^{\infty}+(-1)\int_{0+}^{\infty}[D\,g(s,t)]\,[D^{n-1}\ s^{2n-1}D^{n-1}\ s^{2m+n-1}D^{n-1}s^{-k-m+\frac{1}{2}}\ D^{-n+1}\ s^{-m+k-n+\frac{1}{2}}f(s)]ds$$

The integrated part vanishes by virtue of relations (2.1), (2.3a), (2.3b), (2.3c) and (2.4)

Therefore by repeated integration we obtain

$$I=(-1)^{n}\int_{0+}^{\infty} s^{2n-1}\ [D^{n}\ g(s,t)]\ \Big[D^{n-1}\ s^{2m+n-1}\ D^{n-1}\ s^{-k-m+\frac{1}{2}}\ D^{-n+1}\ s^{-m+k-n+\frac{1}{2}}\ f(s)\Big]ds$$

since the integrated parts vanish at each integration.

Integrating by parts again we have

$$I=(-1)^{n+1}\int_{0}^{\infty}[D\ s^{2n-1}\ D^{n}\ g(s\ t)]\Big[D^{n-2}\ s^{2m+n-1}\ D^{n-1}\ s^{-k-m+\frac{1}{2}}\ D^{-n+1}\ s^{-m+k-n+\frac{1}{2}}\ f(s)\Big]ds$$

since the integrated part becomes zero on account of relations (2.1) (2.3a, b and c) and (2.4)

Similarly integrating by parts repeatedly we get

$$I=(-1)^{2n}\int_{0+}^{\infty}[D s^{2m+n-1} D^{n-1}\ s^{2n-1}\ D^{n}\ g(s,t)][D^{n-2}\ s^{-k-m+\frac{1}{2}}\ D^{-n+1}\ s^{-m+k-n+\frac{1}{2}}f(s)]\ ds$$

$$=(-1)^{n}\int_{0+}^{\infty}[s^{-k-m+\frac{1}{2}}\ D^{n-1}\ s^{2m+n-1}\ D^{n-1}\ s^{2n-1}\ D^{n}\ g(s,t)]\ [D^{-n+1}\ s^{-m+k-n+\frac{1}{2}}\ f(s)]ds$$

$$=(-1)^{2n-1}\int_{0+}^{\infty}[s^{-m+k-n+\frac{1}{2}}\ D^{-n+1}\ s^{-k-m+\frac{1}{2}}\ D^{n-1}\ s^{2m+n-1}\ D^{n-1}\ s^{2n-1}\ D^{n}\ g(s,t)]\ f(s)\ ds$$

for the integrated parts vanish at each integration.

We have using (2.3)

$$s^{2n-1}\ g^{(n)}(s,t)=\frac{\Gamma(2m+1+n)\ \Gamma(1+n)}{\Gamma(m-k+n+\frac{1}{2})}(-1)^{n}\ s^{2n-1}\ t^{-n-1}\ F(2m+1+n\ \ 1+n\ \ m-k+n+3/2\ \ -s/t)$$

and by the use of (2.2)

$$s^{2m+n-1}\ D^{n-1}\ s^{2n-1}\ D^{n}\ g(s,t)=\frac{\Gamma(2m+1+n)\ \Gamma(2n)}{\Gamma(m-k+n-3/2)}(-1)^{n}\ s^{2m+2n-1}\ t^{-n-1}\ F(2m+1+n\ \ 2n\ \ m-k+n+3/2\ \ -s/t)$$

Again using (2.2) we obtain

$$s^{-k-m+\frac{1}{2}}\ D^{n-1}\ s^{2m+n-1}\ D^{n-1}\ s^{2n-1}\ D^{n}\ g(s\ t)=\frac{\Gamma(2m+\ \ \ \Gamma(\ \ )}{\Gamma(m-k+n+3/2)}(-1)^{n}\ s^{m+n-k+\frac{1}{2}}\ t^{-n-1}\ F(2m+2n\ \ 2n\ \ m-k+n+3/2\ \ -s/t)$$

Now by (2.1) we have

$$(-1)^{n}\ s^{-m+k-n+\frac{1}{2}}\ D^{-n+1}\ s^{-k-m+\frac{1}{2}}\ D^{n-1}\ s^{2m+n-1}\ D^{n-1}\ s^{2n-1}\ D^{n}\ g(s\ t)$$

$$=\frac{\Gamma(2m+2n)\,\Gamma(2n)}{\Gamma(m-k+2n+\frac{1}{2})}\, s^{n}\, t^{-n-1}\, F\,(2m+2n,\ 2n;\ m-k+2n+\tfrac{1}{2};\ -s/t)$$

Then

$$\int_{0+}^{\infty} g\,(s,\ t)\ L_{n,s}\ f(t)\ ds = \frac{\Gamma(2n+m+k-\frac{1}{2})}{\Gamma(n+1)\ \Gamma(n+m+k-\frac{1}{2})}$$

$$\int_{0+}^{\infty} s^{n}\, t^{-n-1}\ \ F(2m+2n,\ 2n;\ m-k+2n+\tfrac{1}{2};\ -s/t)\, f(s)\ ds$$

$$\sim \frac{\Gamma(2n+m+k-\frac{1}{2})}{\Gamma(n+1)\ \Gamma(n+m+k-\frac{1}{2})}\int_{0+}^{\infty}\frac{s^{n}\ \ t^{n+m+k-3/2}}{(s+t)^{2n+m+k-\frac{1}{2}}}\, f(s)\ ds\ \ (n\to\infty)$$

since

$$F\,(2m+2n,\ 2n;\ m-k+\tfrac{1}{2}+2n;\ -s/t)\sim(1+st^{-1})^{-m-k+\frac{1}{2}-2n}\ \ (n\to\infty)$$

Now by Theorem 4.3 (Arya 1964) the integral

$$\frac{\Gamma(2n+m+k-\frac{1}{2})}{\Gamma(n+1)\ \Gamma(n+m+k-\frac{1}{2})}\int_{0+}^{\infty}\frac{s^{n}\ \ t^{n+m+k-3/2}}{(s+t)^{2n+m+k-\frac{1}{2}}}\, f(s)\ ds \sim f\,(t)\ \ (n\to\infty)$$

Therefore

$$\int_{0+}^{\infty} g(s,\ t)\ L_{n,s}\ f(t)\ ds \sim f(t)\ \ (n\to\infty)(0<t<\infty)$$

This completes the proof of this theorem.

Theorem 2.2 If the integrals

(2.5) $\displaystyle\int_{0}^{1}[u^{2n-1}\ \phi^{(n-1)}(u)]^{(n)}\ du\ \ (n=1,2,3,\ \ldots)$

all exist then there exists a constant B such that

(2.6) $(-1)^{n}\ \ \phi^{(n)}\ (s)\sim B\ n!\ \ s^{-n-1}\ (s\to 0+;\ n=0,\ 1,\ 2,\ \ldots)$

and

(2.7) $f\,(s)\sim A\ s^{-\lambda}\ (s\to 0+)$

where

$$B=\frac{(2m+1)}{(m-k+3/2)}\ \ \frac{(2m+n-1)}{(m-k-\frac{1}{2}+n)}\,A$$

and

$$\phi(\ )=s^{2m+\ \ -1}\,D^{n-1}\ \ s^{-k-m+\frac{1}{2}}\ D^{-\ \ +1}\ \ s^{-m+k-n+\frac{1}{2}}\ f\,(s)$$

Proof We have (Widder 1941 p. 358)

$$(-1)^{n}\ \ \phi^{(n)}(s)\sim B\ n!\ \ s^{-n-1}\ (s\to 0+;\ n=0,\ 1,\ 2,\ \ldots)$$

Hence

$$s^{-k-m+\frac{1}{2}}\,D^{-\ \ +1}\,s^{-m+k-n+\frac{1}{2}}f(s)\sim\frac{B\ (-1)^{\ \ -1}\ s^{-\ \ m-1}}{(2m+1)_{n-1}}$$

$$(s\to 0+)$$

or

$$D^{-n-1}\, s^{-m+k-n+\frac{1}{2}}\; f(s) \sim \frac{B(-1)^{n-1}\, s^{-m+k-3/2}}{(2m+1)_{n-1}} \qquad (s\to 0+)$$

or

$$f(s) \sim \frac{B(m-k+3/2)_{n-1}}{s\,(2m+1)_{n-1}} \quad (s\to 0+)$$

$$\sim A\, s^{-1} \qquad (s\to 0+)$$

Theorem 2.3 If

(2.8) $\int_{0+}^{S} L_{n,s}\; f(s)\; ds = 0(s) \quad (s\to\infty \;\; n=1\;\; 2 \;\;)$

then

(2 9) $f(s) \sim A\, s^{-1} \; (s\to 0+)$

and

(2 10) $f(s) = 0(1) \quad (s\to\infty)$

Proof We have

$$\int_{0+}^{s} L_{n,s}\; f(s)\, ds = (-1)^{n-1} \frac{\Gamma(2n+m-k+\frac{1}{2})\,\Gamma(2n+m+k-\frac{1}{2})}{\Gamma(2m+2n)\Gamma(2n)\Gamma(n+m+k-3/2)\; n!}$$

$$\int_{0+}^{s} D^{n}\, s^{2n-1}\;\; \theta^{(n-1)}\,(s)\; ds = 0(s) \;(s\to\infty \;\; n=1\;\; 2,\,3 \;\;)$$

which implies (2.5) Hence by Theorem 2.2 we have

$$(-1)^{n}\;\; \theta^{(n)}\;\; (s) \sim B\; n!\, s^{-n-1} (s\to 0+ \;\; n=0\;\; 1\;\; 2 \;\;)$$

and $f(s \sim A\, s^{-1}\; (s\to 0+)$

Also

$$\int_{0}^{s} D^{n}\; s^{2n-1}\;\; \theta^{(n-1)}\,(s)\; ds = 0(s)\;\; (s\to\infty)$$

implies

$$\theta^{(n-1)}(s) = 0\; s^{-n+1}) \quad (s\to\infty) \;\; (n=1\;\; 2 \;\;)$$

or $\theta\; s) = 0(1) \quad (s\to\infty)$

from which we have

$$D^{n-1}\, s^{-k-m+\frac{1}{2}}\; D^{-n+1}\; s^{-m+k-n+\frac{1}{2}}\; f(s) = 0(s^{-2m-n+1})\; (s\to\infty)$$

or $\qquad f\,s) = 0(1) \qquad (s\to\infty)$

which proves the theorem.

Theorem 2.4 If

$$\int_{0+}^{x} L_{n,s}\;\; f(s)\;\; ds = 0(x) \;\; (x\to\infty \;\; n=1\;\; 2 \;\;)$$

and if $f(\infty)=0$ then

$$(2.11)\quad f(x)=\lim_{n\to\infty}\frac{\Gamma(2n+1)}{\Gamma(n-k+3/2)}\quad\frac{1}{x}$$

$$\int_{0+}^{x} F(2n+1,\ 1,\ n-k+3/2,\ -s/x)\ L_{n,s}\ f(s)\ ds+A\ x^{-1}\quad (x\to 0+)$$

where $\quad A=\lim_{x\to 0+}\ x f(x)$

Proof : Let us set

$$g(x)=f(x)-A\ x^{-1}$$

Then

$$g^{(n)}(x)=f^{(n)}(x)-A(-1)^{n}\ n!\ x^{-n-1}$$

or using Theorem 2.3

$$g^{(n)}(x)=o(x^{-n-1})\quad (x\to 0+,\ n=0,\ 1,\ 2,\ \ldots)$$

$$=o(x^{-n})\quad (x\to\infty,\ n=0,\ 1,\ 2,\ \ldots)$$

Hence $L_{n,s}\ g(x)=L_{n,s}\ f(x)-L_{n,s}\ A\ x^{-1}=L_{n,s}\ f(x)$

Applying Theorem 2.2 we obtain (2.11)

Corollary 2.4a If

$$\underset{0=s<\infty}{\text{l. u. b.}}\left|L_{n,s}\ f(x)\right|\ <\ \infty\quad (n=1,\ 2,\ \ldots)$$

and if $f(\infty)=0$ then (2.11) holds.

Corollary 2.4b If for some $p=1$

$$(2.12)\quad \int_{0}^{\infty}\left|L_{n,s}\ f(x)\right|^{p}\ ds<\infty\quad (n=1,\ 2,\ \ldots)$$

then (2.11) holds.

Th proof is similar to that of the corollary proved by Widder (Widder 1941 p. 361)

REPRESENTATION THEOREM FOR STIELTJES INTEGRAL

Definition 3.1 A function $f(x)$ satisfies conditions D if it has derivatives and integrals of all orders in $(0,\infty)$ and if there exists a constant M such that

$$(3.1)\quad \int_{0}^{\infty}\left|L_{n,s}\ f(x)\right|\ ds\ <\ M\quad (n=1,\ 2,\ \ldots)$$

Theorem 3.1 A necessary and sufficient condition that

$$(3.2)\quad f(x)=\frac{\Gamma(2n+1)}{x\Gamma(n-k+3/2)}\int_{0}^{\infty}F(2n+1,\ 1,\ n-k+3/2,\ -t/x)\ d\alpha(t)$$

with $\alpha(t)$ of bounded variation in $(0\ \infty)$ is that $f(x)$ should satisfy conditions D

Proof Let us first prove that the conditions are necessary

We have

$$L_{n,s}\ f(x) = \frac{\Gamma(2n+m+k-\frac{1}{2})}{\Gamma(n+1)\,\Gamma(n+m+k-\frac{3}{2})}\, s^{-n-1}$$

$$\int_0^{\infty} t^n\, F(2m+2n\ \ 2n\ \ m-k+\tfrac{1}{2}+2n\ \ -t/s)\ d\alpha(t)\quad (n=1,2,\ \ )$$

Hence

$$\int_0^{\infty} \left| L_{n,s}\ f(x) \right| ds \le \left| \frac{\Gamma(2n+m+k-\frac{1}{2})}{\Gamma(n+1)\Gamma(n+m+k-3/2)} \right|$$

$$\left| \int_0^{\infty} s^{-n-1}\ ds \int_0^{\infty} t^n\ F\ (2m+2n\ \ 2n\ \ m-k+\tfrac{1}{2}+2n\ \ -t/s)\ d\alpha(t) \right|$$

$$\le \left| \frac{\Gamma(2n+m+k-\frac{1}{2})}{\Gamma(n+1)\Gamma(n+m+k-3/2)} \right| \left| \int_0^{\infty} t^n\ |d\alpha(t)| \right| \left| \int_0^{\infty} s^{-n-1}\ F(2m+2n\ \ 2n\ \ m-k+\tfrac{1}{2}+2n\ \ -t/s)\ d \right|$$

provided that the iterated integral exits.

But

$$\int_0^{\infty} s^{-n-1}\ F(2m+2n\ \ 2n\ \ m-k+\tfrac{1}{2}+2n\ \ -t/s)\ ds$$

$$= \frac{\Gamma(2m+n)\,\Gamma(n)\,\Gamma(m-k+\frac{1}{2}+2n)\,\Gamma(n)}{\Gamma(2m+2n)\,\Gamma(2n)\,\Gamma(m-k+\frac{1}{2}+n)}\ t^{-n}$$

provided that Re $(2m+n)>0$ and $m-k+\frac{1}{2}+2n \neq 0$ or a negative integer

Therefore

$$(\ .3)\quad \int_0^{\infty} |L_{n,s}\ f(x)|\ ds$$

$$\le \left| \frac{\Gamma(\ \ +m+k-\frac{1}{2})\,\Gamma(2m+n)\,\Gamma(n\ \ \Gamma(m\ \ k+\frac{1}{2}+2n)\,\Gamma(\ )}{\Gamma(n+1)\,\Gamma(n+m+k-3/2)\,\Gamma(2m+2n)\,\Gamma(\ n)\,\Gamma(m-k+\frac{1}{2}+n)} \right|$$

$$\int$$

Hence the total variation of $\alpha(t)$ may be taken as M since

$$\frac{\Gamma(2n+m+k-\frac{1}{2})\,\Gamma(2m+n)\,\Gamma(m-k+\frac{1}{2}+2n)\,\Gamma(n)\,\Gamma(n)}{\Gamma(n+1)\,\Gamma(\ \ +m+k-3/2\ \ \Gamma(2m+\ \ )\,\Gamma(2n)\,\Gamma(m-k+\frac{1}{2}+n)}$$

approaches unity as n tends to infinity

Thus the necessity is established.

Next let the condition be satisfied so that

$$\int_0^\infty \left| L_{n,s}\ f(x) \right| ds < M \quad (n=1, 2, \ldots)$$

Then from Corollary 4.2b we have

$$f(x) = \lim_{n\to\infty} \frac{\Gamma(2n+1)}{\Gamma(m-k+3/2)} \int_0^\infty F(2m+1, 1; m-k+3/2; -s/x)\, L_{n,s}$$
$$f(x)\ ds + A\, x^{-1} \quad (x\to 0)$$

where

$$A = \lim_{x\to 0+} x\, f(x)$$

Now let us consider functions

$$\alpha_n(s) = \left| \int_0^s L_{n,t}\ f(x)\ dt \right| \quad (n=1, 2, \ldots)$$

Each function $\alpha_n(s)$ has total variation less than M by (3.2). Hence we have a sequence of functions $\left[\alpha_{n_i}(x) \right]_0^\infty$ taken from the sequence of functions $\left[\alpha_n(x) \right]_0^\infty$ such that

$$\lim_{i\to\infty} \alpha_{n_i}(\ ) = \alpha(x)$$

where $\alpha(\ )$ is a function of bounded variation in $(0, \infty)$

Hence

$$f(x) = \lim_{i\to\infty} \frac{\Gamma(2m+1)}{x\Gamma(m-k+3/2)} \int_0^\infty F(2m+1, 1; m-k+3/2; -s/x)\, d\alpha_{n_i}(s)$$
$$+ A\, x^{-1} \qquad (x>0)$$

$$= \lim_{i\to\infty} \frac{\Gamma(2m+1)}{x\Gamma(m-k+3/2)} \left[\alpha_{n_i}(s)\, F(2m+1, 1; m-k+3/2; -s/x) \right]_0^\infty -$$

$$-\lim_{i\to\infty} \int_0^\infty \alpha_{n_i}(\ )\ (d/ds)\ F(2m+1, 1; m-k+3/2; -s/x)\ ds + A\, x^{-1}$$

$$(-1) \frac{\Gamma(2m+1)}{x\Gamma(m-k+3/2)} \int_0^\infty (s)\ (d/ds)\, F(2m+1, 1; m-k+3/2; -s/x)\ ds$$
$$+ A\, x^{-1}$$

$$= (-1) \frac{\Gamma(2m+1)}{x\Gamma(m-k+3/2)} \left[\alpha(s)\ F(2m+1, 1; m-k+3/2; -s/x) \right]_0^\infty +$$

$$+ \frac{\Gamma(2m+1)}{x\Gamma(m-k+3/2)} \int_0^\infty F(2m+1, 1; m-k+3/2; -s/x)\ d\alpha(s) + A\, x^{-1}$$

$$= \frac{\Gamma(2m+1)}{x\Gamma(m-k+3/2)} \int_0^\infty F(2m+1, 1; m-k+3/2; -s/x)\ d\alpha(s) + A\, x^{-1}$$

But (A/x) can be expressed as an integral of the form (3 2) with $\alpha(t)$ defined as follows

$$\alpha(t) = -\frac{\Gamma(m-k+3/2)}{\Gamma(2m+1)} \quad (t=0)$$
$$=0 \qquad\qquad (t\neq 0)$$

Thus we have proved that the conditions D are sufficient.

This completes the proof of this theorem

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